

nature

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Inequality of opportunity: questions but no answers

THE table shows admissions to UK universities in October 1975 for a selection of subjects. An attempt is made not only to show how many places are available as a percentage of the number of candidates, but also to indicate how some subjects are major exporters, others major importers of students. The medical sciences continue to export, notably to biology and biochemistry, even though the numbers of applicants for medicine was sharply down. The vigour of biology is remarkable. Even though it imported 40% of its students, applicants who named biology as their first preference grew by

20% from the previous year. Biomedically inclined schoolchildren are probably being increasingly advised to apply for biology rather than medicine. The physical sciences continue in the doldrums.

However much a popular subject, such as medicine or veterinary science, may export, it is abundantly clear that many students with quite good qualifications are being turned away who would have been accepted with open arms if they had possessed the same qualifications in less popular subjects. Can we afford to do this? How could it be stopped?

Subject	Candidates preferring this subject (a)	Places filled in this subject (b+d)	Percentage imported 100d/(b+d)	Percentage exported 100c/b	Percentage of candidates who get in somewhere 100(b+c)/a	Places per candidate (b+d)/a
Veterinary science	1,467*	278†	1	136	44	0.19
Medicine	12,046††	3,208	1	67	44	0.27
Architecture	2,345	640	3	25	33	0.27
Dentistry	2,969†	898	14	45	38	0.30
Pharmacy	2,679	818†	14	41	37	0.30
Law	8,351	3,200	4	25	46	0.38
Civil engineering	4,818*	2,422	13	14	50	0.50
Geography	4,128	2,098	9	18	54	0.51
Psychology	2,570	1,337†	17	19	51	0.52
Mechanical engineering	3,350*	1,789	17	12	50	0.53
Music	1,109**	609	3	4	55	0.55
English	5,666	3,177*	12	25	62	0.56
Electrical engineering	4,392	2,537	13	10	55	0.58
Sociology	2,820†	1,679	29	24	53	0.59
French	1,777†	1,217††	25	26	64	0.68
Biology	2,461****	1,714*	41	36	56	0.70
History	3,805	2,572	13	18	69	0.71
Economics	3,107**	2,233**	37	30	59	0.72
Mathematics	4,595†	3,395††	10	8	71	0.74
Physics	2,295††	1,990††	22	12	76	0.87
Classics	517	485†	12	5	87	0.94
Chemistry	2,292	2,211	30	7	73	0.96
Biochemistry	1,107	1,076†	52	25	58	0.98
ALL SUBJECTS (many not listed above)	131,478	71,211	—	—	54	0.54

a = No. of candidates preferring this subject.

b = No. of candidates preferring this subject and accepted for it.

c = No. of candidates preferring this subject and accepted for another.

d = No. of candidates preferring another subject and accepted for this one.

Each * represents a 5% increase in numbers over 1974 (the global upward trend having been removed).

Each † represents a 5% decrease in numbers over 1974 (the global upward trend having been removed).

SOURCE: Thirteenth Report of Universities Central Council on Admissions (UCCA).



Controlling the new proliferation

Trade in nuclear technology is increasing. John Stares considers whether the nuclear Non-Proliferation Treaty, six years old last week, can still effectively control the spread of nuclear weapons.

DURING the six years since the Treaty on the Non-Proliferation of Nuclear Weapons (NPT) came into force, the peaceful uses of nuclear technology have become much more widespread. With this development has come the increased risk of the misuse of nuclear technology, for military rather than peaceful purposes. Through peaceful nuclear programmes, many countries (in addition to the five nuclear-weapon powers) have accumulated the technical expertise and knowledge, as well as the fissile materials, necessary to develop and manufacture nuclear weapons. In time the number of such countries will grow, and some disarmament experts have predicted that within a few years more countries will in fact "go nuclear". The Indian nuclear test in the spring of 1974 clearly demonstrated that, even for an underdeveloped country, the technological and economic constraints on producing nuclear explosives (whether for peaceful or military ends) that existed a few years have largely disappeared. Is it possible, then, to prevent nuclear-weapon proliferation? If so, is the NPT an effective means of doing so? Or must other means be found?

It is certain that the global demand for energy will continue to grow, and that the rate of growth will be highest in the underdeveloped countries. Development requires a plentiful supply of energy—cheap energy—and in the face of shortages and rising prices of other energy sources (oil, for example) the only viable alternative energy source at the moment for many, if not most, underdeveloped countries is nuclear energy. The capital outlays for constructing nuclear power stations may be higher than for oil- or coal-fired power stations, but nuclear fuelling costs are considerably lower than conventional ones, thus making nuclear electricity generation at least competitive in terms of cost with other methods. This is well illustrated by the fact that Iran plans to produce much of its future energy from nuclear power stations, even though it has enormous oil reserves: it is more economical for Iran to sell or conserve its oil than to burn it in power stations.

It is therefore right and proper that nuclear energy should be made available to the underdeveloped countries. This was recognised in the NPT: the Preamble declares that "the benefits of

peaceful applications of nuclear technology should be made available for peaceful purposes to all Parties to the Treaty". But any country with its own nuclear power reactor has, on its territory, the raw materials for making nuclear weapons. (It is interesting to note that if a power reactor is operated to produce the cheapest electricity, the plutonium it produces will consist of a mixture of isotopes and will be suitable for making only crude nuclear weapons, not optimally efficient ones.) The number of countries with nuclear programmes is increasing. In 1970 when the NPT became effective, 15 countries were operating power reactors. By 1975 the number had risen to 19. And if present plans are fulfilled, nearly 30 countries will have their own nuclear power stations by 1980. The increase in the number of actual reactors is even more dramatic. In 1975 about 170 power reactors were in operation, about two-thirds of them in the nuclear-weapon countries. By 1980 it is estimated that the total will have increased to more than 400, and that more than half will be in countries that do not, now, have nuclear weapons.

In theory, the NPT seemed a reasonable system for ensuring that the spread of peaceful nuclear technology did not lead to the proliferation of nuclear weapons. Under the Treaty, all non-nuclear-weapon countries undertook not to manufacture or otherwise acquire nuclear weapons, or indeed any nuclear explosive device, and to permit the application of control procedures, called safeguards, on its nuclear activities "with a view to preventing the diversion of nuclear energy from peaceful purposes to nuclear weapons or other nuclear explosive devices". All parties to the Treaty undertook not to supply fissile material or equipment designed for processing, use or production of fissile material to a non-nuclear-weapon country without safeguards being applied. The Treaty certainly has its weaknesses, perhaps the major one being that it is imbalanced in favour of the nuclear-weapon countries. These were left free to develop and increase their own nuclear arsenals, and were not obliged to submit to safeguards on their nuclear activities.

The Treaty therefore does not ensure the non-proliferation of nuclear weapons, but only seeks to stop their spread to non-nuclear-weapon coun-

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Can anyone prevent this . . .

tries. However, if *all* countries had joined the NPT, the nuclear proliferation problem might now be much less serious than it is: an international system of safeguards covering all nuclear activities (except in the nuclear-weapon countries) would be in operation, and all but five countries would have given up the option of producing nuclear weapons. But several important countries are still not members. This group includes two nuclear-weapon powers, France and China; several other industrial countries, such as Japan (which signed the Treaty in February 1970 but has still not ratified it); and many underdeveloped countries, India being the classic example and others including Brazil, Egypt (which has signed, but not ratified), Israel, Pakistan and South Africa. Many of the countries, industrial and underdeveloped, that remain outside the Treaty are already sufficiently advanced technologically to be able to produce their own nuclear weapons very quickly if ever they decide to do so. It is even believed that some may secretly have done so.

The nuclear exporting countries are clearly worried by this situation and by the possibility that future exports of nuclear materials and equipment might be diverted to military uses. There have been protests at nuclear exports in a number of countries: for example, in the United States there were calls during recent hearings of the Senate Government Operations Committee for an embargo on nuclear exports, and in the

Canadian Parliament the Opposition has criticised the Government for allowing economic interests to take precedence over more serious concerns about proliferation. Despite these protests, it is unlikely, for economic reasons, that exports will stop. The nuclear-weapon countries have invested huge sums in developing nuclear technology, and they and other industrial countries such as Japan and West Germany look to exports to boost their economies. The United States is reported to earn about \$1 billion per year from its nuclear shipments; the West German deal to supply Brazil with a complete fuel cycle is worth some \$5 billion; and the recently announced deal between Canada and South Korea for the supply of a 600 MW reactor involves Canadian, British and US loans totalling \$400 million.

Concern over misuse

But although the nuclear exporting countries may be unwilling to halt exports, they are concerned to prevent the misuse of exported technology. During 1975, seven of them—the so-called Nuclear Exporters Group consisting of Canada, France, West Germany, Japan, the USSR, the UK and the USA—held a series of secret meetings on this issue. In January this year they were reported to have reached agreement on new safeguards and control procedures. The exact details remain secret, but according to Dr Fred Ikle, Director of the US Arms Control and Disarmament Agency, in testimony to the Senate Foreign Relations Subcommittee on Arms Control, it is thought that in future, requirements for nuclear exports will involve: acceptance by recipient countries of internationally accepted safeguards drawn up by the International Atomic Energy Agency; assurances by recipient countries that they will not use nuclear exports to make nuclear explosives, for any purpose; provision by importers of adequate security measures to prevent theft or sabotage; and assurances by importers not to transfer materials or duplicates of equipment to third parties without setting similar conditions.

The effect of such measures is not likely to be striking. They may be seen as an attempt to strengthen the NPT, since they are, in essence, similar to the provisions contained in the Treaty itself. In the past, disarmament experts have suggested that membership of the NPT should be demanded as a requirement for receiving nuclear assistance. Apart from the fact that some countries may regard such a move as even more restrictive and discriminatory than the NPT itself, the Group could hardly adopt such a policy when two of its members have not themselves joined the Treaty. The measures could thus

be regarded as a “second best” answer to this suggestion. On the other hand, it is even possible to envisage that these measures might weaken, rather than strengthen, the NPT: countries that have not already joined the Treaty may now perceive that there is no pressing need to do so, since they know that the exporting countries are willing to supply assistance under terms that are similar to those of the NPT—but with the important difference that recipient countries will only be required to make a “gentleman’s agreement” not to make nuclear weapons, which is very different from undertaking a formal international treaty obligation not to do so.

What must be accepted sooner or later is that it is simply not possible to *prevent* a country from acquiring the *potential* to produce nuclear weapons. Applying stringent controls or conditions may slow the process, but any country with a peaceful nuclear programme will inevitably acquire a nuclear-weapon potential in the end. What may be possible—in fact what may be the only answer—is to *discourage* countries from realising this potential. The only barrier left to the acquisition of a nuclear force, now that the technological and economic constraints have lost their importance, is a political one. For countries that have joined the NPT, this Treaty represents the barrier: to produce nuclear weapons would be a violation of international commitments. Once countries that have not joined the Treaty acquire a nuclear-weapon potential, they will have the choice whether or not to

realise it. This choice will be influenced by political, not technological, considerations, principally the countries’ perceptions of their security needs.

The key here is the attitude and behaviour of the nuclear-weapon powers themselves. If they indicate that they do not regard nuclear weapons as essential to security by showing themselves willing to make meaningful reductions in their nuclear arsenals, other countries might well be encouraged to follow their example, and renounce the nuclear option. In Article VI of the NPT, all parties undertook “to pursue negotiations in good faith on effective measures relating to the cessation of the nuclear arms race at an early date and to nuclear disarmament, and on a treaty on general and complete disarmament under strict and effective international control”. It can hardly be said that the nuclear weapon powers have lived up to this commitment: the United States and the Soviet Union have been engaged in the Strategic Arms Limitation Talks (SALT) for years, but they have reached no agreements of any significant disarmament value, and the build-up of their nuclear arsenals continues unabated; Britain has also continued to develop its nuclear force, as have France and China, which have not even joined the NPT. An act of faith on the part of these powers, a demonstration that they are serious in their intention to abide by their treaty obligations and in their attempts to achieve nuclear disarmament, would have vastly more impact on world peace and security than any number of secret meetings. □

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... being developed from this?

SPACE

Bringing cooperation nearer

A joint move backing planned European involvement with a major NASA space science project of the 1980s has come from the European Science Foundation and the US National Academy of Sciences. Allan Piper reports

FOLLOWING a meeting between the European Science Foundation and the US National Academy of Sciences at Williamsburg in Virginia, it has become clear that the independent scientific community represented by the two organisations means to become involved in the new \$350-millions Large Space Telescope project (LST) planned for the 1980s by NASA. Recommendations developed during the meeting particularly underline European interest in the telescope, already under consideration as a priority project in the European Space Agency's programme of co-operation with NASA.

The LST, planned as a 2-metre diffraction limited optical telescope, should be in orbit by the end of 1983 as part of NASA's forthcoming space shuttle programme. Essentially an advanced and updated version of the highly successful Copernicus telescope launched four years ago, it will be used in planetary, galactic and intergalactic studies. The planned payload includes instrumentation covering the entire optical spectrum from infrared to ultraviolet, providing astronomers with a first opportunity to obtain data which is not accessible from Earth-

bound observatories.

Described at Williamsburg as the single most important next step that could be taken in astronomy, the project hogged the spotlight during the wider debate on international cooperation on space observatories.

Recommendations resulting from the meeting call among other things for an independent, international LST Science Institute. An ESF-USNAS advisory committee is also suggested to oversee the selection of instruments for the telescope's scientific payload.

But while the recommendations outwardly stress the importance of independent scientific involvement, it is the implicit backing for ESA-NASA cooperation on the project which is more significant. None of the Williamsburg scientists was directly involved with the LST, but their statements, both space agencies have indicated, will weigh in decisions on the next moves.

Under the circumstances that could prove crucial. The project is running through heavy weather in the US, where Congress has just declined to approve NASA funding for 1977 in the absence of outside backing. The congressional coyness is seen as nothing more than a firm come-on to ESA, known to be close to throwing in its lot for a share of the price tag, but there is a very real danger that it will serve only to turn away a possibly faint-hearted suitor.

The problem is that Congressional and ESA decision-making are almost exactly six months out of phase. So

even though there is confidence that Congress will give NASA the nod for 1978 funds if ESA adopts the project in the meantime, ESA officials who were in any case sceptical of the project's running power in competition with the other five possible cooperative space ventures will now have to be convinced additionally that it is not a complete non-starter.

Before losing heart, however, ESA can reflect that Congress may view the project more favourably once the present heavy expenditure on the space shuttle begins to run down. Moreover, there is still a glimmer of hope that the decision not to programme 1977 funds for the LST may be reversed. In the US the Congressional snub has brought a deluge of angry protest letters from astronomers and other interested scientists. There is little chance at this stage of additional funds, but Congress is holding open an option on internal reallocation.

Although the European astronomy community is not as well organised at an informal level as its American counterpart, the need for a tightly orchestrated campaign before, and not after, an ESA decision is widely recognised. Already there is European concern that ESA's option on the project will go by the board because of misguided views that it will not serve any immediate needs in astronomy over and above those provided by projects already in the pipeline, such as the Northern Hemisphere Observatory. It is at that level that the ESF Space Science Committee, which shares observers with ESA, will be most effective in pointing out that the LST offers new and unique possibilities, and is not just a superfluous bonus. □

COMECON

THE new Five Year Plans of the Comecon member countries began this year, and according to the Comecon Committee on Scientific and Technical Cooperation, which met in Budapest in January, scientific cooperation will be largely devoted to research projects and problems "which cannot be solved by individual countries". These include power production, protection of metals against corrosion, increase of protein production, the comprehensive utilisation of timber, and the testing of new insecticides. Discussions will also take place on the establishment of an integrated system for the exchange of scientific and technical information which, if successfully implemented, could eli-

minate uneconomical duplication of research.

Special emphasis is to be placed on research into energy resources ranging from lignite to nuclear power. The production and consumption of energy by the Comecon bloc is still steadily expanding: according to Nikolai Faddeev (USSR), the Secretary of Comecon, the nuclear power stations in the USSR and those built with Soviet assistance in Bulgaria, the GDR and Czechoslovakia now have an aggregate capacity of 7,500 MW, and by 1980 this figure will have risen to some 30,000 MW.

A 2,750 km pipeline from Orenburg to the western frontier of the Soviet Union will carry some 15,000

million m³ of gas per year from the Orenburg gas condensate deposit to Bulgaria, Hungary, the GDR, Romania and Czechoslovakia. The second string of the *Druzhba* oil pipeline from the Soviet Union has been completed, and in 1975 delivered some 50 million tons of oil. Joseph Zamparo, of the University of Genoa, addressing the Conference on Energy Alternatives sponsored by the American Nuclear Society, said that energy consumption of the Communist countries (including China) was increasing by an average of 6.5%, whereas all other countries were reducing their energy consumption by amounts ranging from 9% for Western Europe to 2% for Australia.

USA

A \$20-million boost for earthquake prediction studies hangs in the balance at the moment, following reluctance on the part of the Office of Management and Budget to sanction the extra spending. Much of the initiative in seeking out the extra money has come from Vice-President Nelson Rockefeller, who has been particularly active in support of extra funds for earthquake research since the disaster in Guatemala.

All is not lost, however, as proponents of earthquake research were testifying before a Congressional committee last week and pointing out, in particular, implications of the recent finding that a small part of California has uplifted very recently, by as much as 25cm in 10 years.

If the increase of \$20 million were to be given the go-ahead, it seems likely that it would be spent not on the employment of additional personnel by the US Geological Survey but on research in private industry and universities. The programme would include work on the socio-economic aspects of earthquakes and their prediction, on the effects of earthquakes and on an effort to develop within a decade the capability to predict earthquakes satisfactorily.

The scheme is, however, "comatose" at present, though with luck and some judicious Congressional lobbying it may yet get off the ground.

- More evidence of the "drug lag" in the United States—whereby the Food and Drug Administration now takes so long to give permission for clinical trials and ultimately for the sale of a drug that some key preparations are available in the USA long after they go on the market elsewhere in the world—is contained in a recent report showing how and where the US pharmaceutical industry spent its money on research and development.

Figures released by the Pharmaceutical Manufacturers Association (PMA) recently indicate that of the \$859 million spent by US drug companies on research and development in 1974, \$133 million was spent outside the USA. The latter amount is 22% higher than the figure for 1973, which in turn was 66% higher than for 1972. The amount spent within the United States was 12.8% higher in 1974, at \$726 million. The increases for 1975, as budgeted, were more modest, 12% for spending outside the United States and 10% for spending within the country.

The PMA points out that, on average, it now costs \$11.5 million to develop a drug inside the USA and \$7.5 million outside, and that whereas

US companies had accounted for some 70% of the new chemical agents put on the market between 1940 and 1970, that proportion divided to some 39% between 1971 and 1973.

The PMA emphasises that it does not want the drug regulations to be relaxed, but simply to see the FDA become more efficient and responsive to the need to get new drugs on to



Photo: Cam & Pen Int

the market as quickly as possible. In 1972 it took between 5½ and 8 years to get a drug passed by the FDA, by contrast with the situation elsewhere in the world where the comparable period of time, the association maintains, was 6 to 24 months.

Perhaps Senator Edward Kennedy's proposal to split up the FDA into two separate agencies and streamline the procedures for drug approval would help matters.

- The Nuclear Regulatory Commission (NRC) has been served up with a critical but constructive report by the Special Review Group it set up to look into the celebrated fire at Browns Ferry nuclear power station, Alabama, last year. The fire was started by a candle being used to check for air leaks around cables passing from one of the two reactor buildings to a cable spreading room beneath the control room.

The report points out that the inflammable polyurethane foam used to block off air draughts was not in the original design for the plant, and, like the candle, should not have been used. The group recommends that the foam should either be replaced wherever it has been used in the Browns Ferry or other nuclear plants, or that special safety procedures should be set up if this is not possible. More generally, the group says that, better all-round fire prevention measures are needed in nuclear plants.

Fire fighting procedures at the Browns Ferry also come in for criticism. The report points out, in particular, that the part of the fire in the reactor building "was fought unsuccessfully for several hours with portable CO₂ and dry chemical extinguishers; however, once water was used, it was put out in a few minutes". The old maxim about not using water on electrical fires has to go quickly by the board in such cases if other means clearly fail, the report concludes after considering the views of fire experts.

The fact that several of the plant's control systems were damaged in the fire, including redundant systems that should have prevented complete loss of any function, also led the group to criticise other features of the Browns Ferry design (which, to be fair, was carried out in the mid-1960s). For example, wiring connected to some redundant devices was all located close together, with one set simply placed in a conduit. In spite of this, the fire damaged both sets of wires in several instances.

The group recommends that the NRC should sharpen up many of its procedures for developing standards, making inspections and so on, but points out that the very small probability of accidental release of radioactivity from such a fire is "about 20%" of that calculated from all other possible causes. Thus "urgent" action to reduce fire risks is not required, it says, and in particular there is no question of shutting down other plants while minor modification work is carried out.

The Browns Ferry reactors are expected to start generating electricity again in April.

- A paper released by the Washington-based Worldwatch Institute (*Abortion Liberalisation: A Worldwide Trend*) reports that 64% of the world's people live in countries where abortion is liberally available. Five years ago, in January 1971, the proportion was 38%, and writers Lester R. Brown and Kathleen Newland say that "few social changes have ever swept the world so rapidly".

The worldwide movement, they argue, "reflects an increasing willingness by national legislatures to face the reality of abortion as a public health issue"—but the public health benefits of safe abortion depended on its availability in fact as well as in legal theory, which made cost and distribution important. Women would also continue to seek abortion, even when illicit and dangerous, while there was a lack of failure-proof and problem-free contraceptives.

FRANCE

THE agreement reached at the recent Nice summit between the French and West German Technology ministers on cooperation in nuclear reactor technology, while vague, represents a pooling of risks, regarding both fast breeders and high temperature reactors, that both countries hope will work to their mutual advantage.

The indications are that Germany will benefit in the sphere of fast breeders. Her operation, conducted along with the Benelux countries, is racked by rising costs and due to bear fruit only in 1981, while France's 250 MW Phenix prototype sodium-cooled reactor has been working successfully for a year. In the field of high temperature reactors, on the other hand, Germany's project, although also behind schedule and increasingly costly, is one of the few of its type, and the Schmidt government appears prepared to take it further.

The money involved, of course, is enormous, and it is not clear whether financial backing will be divided equally between the two areas. It is expected that the two countries will contribute equally, but the agreement declares only that they should spend the amounts on the two areas taken together.

The conclusion came just a few weeks after the completion of a deal between France and the Westinghouse Electric Corporation which also reflected a trend to "Europeanise" the nuclear industry this side of the Atlantic. Westinghouse is to sell two-thirds of its 45% holding in Framatome, the French licensee of its pressurised light-water reactor, to the French Atomic Energy Commission (CEA) for \$25 millions.

Westinghouse will transfer its remaining 15% Framatome interest to the Creusot-Loire Company, the heavy equipment suppliers with a 51% interest in Framatome, in 1982, when the licenses expire. Until then, Westinghouse will be able to buy up to 1,200 tonnes of uranium from the CEA. Westinghouse will also continue to receive royalties for Framatome's use of Westinghouse technology in fulfilling orders and options, which now extend to over 40 plants. Westinghouse will take part in a joint research and development programme as well.

This "partnership" through Framatome between the CEA and Creusot-Loire in respect of pressurised-water reactors could conceivably expand into the field of fast breeders with the Franco-German agreement. Cooperation between the two countries on fast

breeders, it is understood, will take place through a joint company. On the German side will be Kraftwerke Union or a subsidiary; on the French side will be the CEA, or possibly Novatome, which is owned by the CEA and Framatome together with the Compagnie Generale d'Electricite (CGE). The CGE was involved in the ill-fated boiling-water reactors about which the government last year changed its mind; but, more im-



Photo: Cam & Pen Int

portantly, it has a stake in a company working on the French fast breeder, GAAA, in which Creusot-Loire has been interested in order to widen its arena of nuclear activity. The size of the respective stakes appear to make it possible for CGE resistance to Creusot-Loire to be circumvented through Novatome.

● Another important deal which, like that involving Westinghouse, took months to finalise, was completed just before the New Year. An agreement between the American computer firm Honeywell Information Systems and the French government merges Honeywell's French subsidiary with the general-purpose business of the national computer company, CII, to form CII-HB, 20% owned by the government and, altogether, 53% owned by French interests.

The government is expected to give about \$280 millions of subsidies over four years, and a government-sponsored finance company will purchase computers sold to the government and nationalised industries. Furthermore, the addition of the 1,300 employees of the CII Research and Development Engineering Department will enhance the research resources of the new company. The whole deal represents the third attempt in eight years to establish a French computer industry that can compete with IBM in the country. But it also represents a setback for attempts to forge an overall European solution.

● The 15-year period after 1955, an era of strong growth in research which coincided with considerable expansion of the French university system, has been followed by a recession which has left university research facing very serious problems.

Now, following the creation of an autonomous National Secretariat for Universities in 1974, ostensibly to oversee both CNRS and university-financed research, the government has belatedly laid some foundations for organising university research policy. A central role has been accorded to the Secretary of State for the Universities (Mme A. Saunier Seit , who replaced M J. P. Soisson in January's ministerial reshuffle) in overseeing co-ordination of university research—an objective that has been sought for a decade. Groups have been formed within the National Secretariat for this purpose, but the overall effort is being directed towards refining budgetary procedures that permit healthy growth as well as adjudicating between university research programmes.

The driving force behind all basic research is the National Scientific and Research Centre (CNRS), with a budget (in 1976) of about 21,000 million francs. It directly finances the major part of research in universities, with which its links are close; the universities' own budget for research last year amounted to an estimated 1,400 million francs, a figure which includes part of the salaries of professors and assistants.

University research which is independent of the CNRS faces typical problems. Laboratories unaided by the CNRS have seen the buying power of their finances eroded since 1969. Moreover, insufficient coordination at the national level has helped to diminish the credibility of an often vague (even non-existent) university research policy. Research teams in the smaller and medium-sized provincial universities have also faced difficulties attaining the necessary size, although all the universities are now feeling the crisis, including such large Parisian universities as the Pierre and Marie Curie University.

● At the end of January, just a fortnight after the court established by France and Britain began considering demarcation between their off-shore oil prospecting limits, on which a decision is expected in the summer, reports emerged of hydrocarbon finds at a depth of 5,840 feet off the Brittany coast, though not in a disputed area. Results of commercial tests are awaited, but drilling will continue to 9,850 feet.

IN BRIEF

Geothermal energy

Geothermal energy in the United Kingdom is not likely ever to be a major resource. This was the unsurprising conclusion to be drawn from evidence recently given to the Energy Sub-Committee of the Commons Select Committee on Science and Technology by Dr Bill Bullerwell, Deputy Director of the Institute of Geological Sciences. Even the few prospects worth pursuing, particularly in Cornwall and the West Hampshire Basin, did not warrant more than a proposed £800,000 research programme over the next three years. In the longer run Dr Bullerwell thought there were possibilities in disused North

Sea wells, where temperatures up to 160 °C have been recorded; water might be circulated and the heat converted to hydrogen storage.

UK energy policy

The UK Department of Energy is aiming for a bigger Government say in shaping the country's future energy policy. The changes it proposes come in a 20-page report circulating among Government and trades union officials which was prepared by the political advisers to the Energy Secretary, Mr Anthony Wedgwood Benn. The report follows his call last week for a new approach to long-term planning, and

suggests the introduction of new administrative set-ups within the nationalised fuel industries.

Telecommunications research

The UK telecommunications industry entered the telephone centenary week with a sharp reminder of its "competitive weaknesses". The Labour Research Department, an independent, union-backed organisation, has suggested that the fault lies in the comparatively low levels of telecommunications research spending in Britain, and sees one possible remedy in the extension of state control to the private supply industries.

MAN has modified the natural landscape in all parts of the world, to make it more productive and to support a larger human population. He has felled the forests which originally covered most temperate areas, and he has drained marshes and fens to produce fertile farmland. In the past these activities were almost universally applauded. In the seventeenth century the Dutch engineer Vermuyden introduced scientific land drainage to East Anglia; he was hailed as a benefactor for turning the almost unproductive wasteland into some of the best arable in England. Others who extended his work, including the drainage of Whittlesea Mere, the largest lake in Southern England, were seldom criticised for their actions. Almost everyone thought that agricultural improvement should be encouraged.

The only objectors to drainage were the few "fen tigers", poor peasants who made their living catching eels and wildfowl, and cutting reed for thatch. Today, when most of Europe's marshes have been drained, and development is destroying (or improving) wetlands throughout the world, the ecological importance of such areas is being increasingly realised. We have the international "Convention on Wetlands of International Importance", and this year, 1976, the Council of Europe is sponsoring a European Wetlands Campaign. The intention is to try to preserve as many as possible of the remaining and unpolluted rivers, estuaries, lakes, ponds, marshes, bogs and fens as habitats for wildlife, particularly for waterfowl.

Agriculture is perhaps no longer the main cause of wetland destruction, though the unwise use of pesticides on crops near to rivers and marshes has taken its toll of fish and fish-eating birds. Industrial pollution has also had its effect on all forms of

wildlife. Marshes near to human settlements, particularly in the tropics, are blamed for breeding mosquitoes which transmit malaria and other diseases, and may be saturated with DDT and other chemicals. Uncontrolled shooting has affected bird populations, and obtrusive sports like water skiing and speedboat racing have turned peaceful lakes into noisy infernos.

Saving the wetlands**KENNETH MELLANBY**

It would be absurd to pretend that the majority of the population of any developed country cared greatly for wetlands and their flora and fauna, and public concern in developing countries must be minimal. So the conservationists must be congratulated in obtaining such widespread international support, from scientists and politicians, for their preservation. However, though many of our governments pay lip service to this type of conservation, their actions have been incredibly dilatory and have not always accorded with their public protestations.

Although there have been inter-

national discussions on wetland and waterfowl conservation for decades, it was only in February 1971 that the text for a convention was formulated at Ramsar in Iran. It was then agreed that when seven different countries had ratified this convention, it would come into force. Bureaucracy has delayed this ratification for five years, during which period many valuable wetlands which might have been saved have been lost.

This delay has been particularly discouraging for the British ornithologists and conservationists who played a leading part in the whole campaign. They hoped that Britain would set an example by rapid action, so leading the world officially as well as scientifically. This was not to be. The convention was first ratified by Australia, then by Iran, Finland, Norway, Sweden and South Africa. This was the state of play in March 1975. One more signature was needed—surely this would be that of Britain. At a meeting in Germany months earlier the Foreign Office had assured our delegation that ratification was "imminent". However, Greece acceded in August 1975, bringing the convention into force. Bulgaria signed next, Britain came in ninth. Now Britain is used to seeing its representatives coming in far down the line at the Olympic Games, so perhaps we should not have been surprised to find our diplomats are as slow as our athletes.

However, let us be thankful for small mercies, in that, at long last, the Foreign Office has given its support to the efforts of our scientists. No doubt the official responsible will be suitably rewarded. As he has been concerned with fens and bogs, the most appropriate honour would be the prestigious Royal Victorian Chain; this rarer decoration would clearly be more suitable than the commoner Order of the Bath.

correspondence

Financial discrimination

SIR,—As is shown by a survey I made recently, reported on by May (February 12, page 446), many journals discriminate in various ways against the more or less impoverished. I publish a journal myself and have grounds for believing that such discrimination is unnecessary. It preserves showiness (at the expense of economies which leave the scientific content intact) and profit. Editors of journals are usually not impoverished, and the present system does have real advantages to those with money.

As with other socially irresponsible powers, a remedy requires joint action. I am therefore pursuing the following actions, and call on others to do likewise:

- To submit no paper to an irresponsible journal except when there is no reasonable alternative.
- To avoid citing papers published after the end of 1976 in irresponsible journals, when alternative citations are available.
- To refrain from refereeing papers for irresponsible journals.

Some journals are more irresponsible than others, and one can be more stringent with the worse ones.

Yours faithfully,

LEIGH VAN VALEN

*Department of Biology,
University of Chicago,
Chicago, Illinois 60637, USA*

Sex laws and inheritance

SIR,—The sexual discrimination laws now in force in the UK should not blind us to the existence of one social inequality which has a sound physiological basis. I mean the nature of inheritance.

In general, any one of our chromosomes (or any part of one) may have come from any one of our grandparents: no fragment of genetic material can be identified with a particular grandparent, with the unique exception of the Y chromosome in men. A man's Y chromosome is certainly derived from his father and from his paternal grandfather and so on. No other chromosome has this singular property.

Consider in particular a woman's X chromosomes. They assuredly were derived one from her mother and one from her father. But the maternal one—is it in turn from her maternal

grandmother or grandfather? It could be from either. Her paternal X chromosome is certainly derived from her paternal grandmother but its pedigree similarly stops short.

Quite generally, any of our forebears, from our grandparents back, may be completely unrepresented in our chromosomes (although crossing-over reduces the probability of any such omission).

Therefore, if a woman and her husband intend only their (genetic) descendants to receive their name, title or family silver, they had better ensure that it goes only to their sons and their sons' male descendants.

Yours faithfully,

J. R. JOHNSTONE

*Department of Physiology,
University of Western Australia,
Nedlands, Western Australia*

Journal guidelines

SIR,—As Professor Ziman remarks (January 29, page 264), evidence for or against the anonymity of referees is difficult to present. However, my claim that six years editorial experience confirmed the validity of the initial policy statement in the *Journal of Aerosol Science* can be enlarged on. There were three reasons for identifying referees:

- The authoritarian age is over. Professor Ziman is in error in identifying this as a populist rejection of constraints imposed by the community. It refers, of course, to constraints imposed by a favoured section of the community.

- The standard of critique will be raised. By eliciting specific criticisms in place of diffuse; by 'curbing the vanity and pride of those who claim authority'; by disclosing a possibly partisan opinion in a debated field; by revealing a possible error in referee selection.

- Encouragement and benefit will result to authors. The young scientist may be put in touch with a well-known figure; an argument between equals becomes definable; a senior worker should know the source of critical comment, especially when it comes from a younger person.

The opposing view of Professor Ziman rests largely upon emotional factors. I do not believe that they are exacerbated by disclosing a personal target—rather the reverse. One would expect a referee to excuse himself if asked to deal with the work of a friend

or rival were the editor ineptly to place him in an embarrassing situation.

Yours faithfully,

C. N. DAVIES

*University of Essex,
Colchester, UK*

Fruit flies as security officers

SIR,—Trained dogs have an established role as aids to Customs Inspectors and Airport Security Guards because of their ability to "smell out" concealed drugs or explosives or other forms of contraband. What seems to have been overlooked is the possibility of using insects for the same purpose.

The simpler insects have genetically fixed responses to specific odours, usually resulting in a conspicuous clustering in the vicinity of the odour source which may be of an extremely low absolute intensity. Kikuchi (*Jap. J. Genet.*, 48(2), 105; 1973 and *Nature*, 243, 36; 1973) isolated a mutant strain of *Drosophila melanogaster* that was strongly attracted to eighteen chemicals which were repellent to the parent strain. It seems likely, therefore, that a systematic search for other strains selectively responsive to various narcotic or explosive emanations can uncover specific "olfactory mutants" of a practically useful sort.

While there would be obvious objections to using such species as cockroaches bees or houseflies, the relatively inconspicuous but not unfamiliar fruit flies would probably be an acceptable alternative to some of the existing security checks.

Yours faithfully,

R. H. WRIGHT

*6822 Blenheim Street,
Vancouver V6N 1R7, Canada*

The JPU in modern science

SIR,—In view of the current interest in citation-counting and other attempts to quantify scientific achievement, I wish to draw attention to a simple concept which may grow in importance in the future. This is the JPU or "Just Publishable Unit" which may be defined as the smallest amount of information which is normally accepted for publication as a separate item in a scientific journal. An example is the present communication.

Yours faithfully,

D. FRASER

*Ministry of Natural Resources,
Maple, Ontario, Canada*

news and views

GENETIC evidence that avian sarcoma viruses carry a specific gene for neoplastic transformation of the host cell has steadily accrued over the past five years. Martin (*Nature*, **227**, 1021; 1970) first isolated mutants of Rous sarcoma virus (RSV) which were temperature-sensitive for maintaining the transformed state of the cell, but which were not temperature-sensitive for viral replication. Then Vogt (*Virology*, **46**, 939; 1971) and Martin and Duesberg (*Virology*, **47**, 494; 1972) observed the spontaneous segregation of transformation-defective variants of RSV that were subsequently shown to be deletion mutants (Lai *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2266; 1973) lacking 10–20% of the viral genome. Recent genetic analysis indicates that all temperature-sensitive transformation mutants represent a single gene (Wyke, Bell and Beaman, *Cold Spring Harbor Symp. quant. Biol.*, **39**, 897; 1975) which map in the region deleted from the transformation-defective mutants (Martin and Bernstein, personal communication). Thus avian sarcoma viruses appear to carry a gene (called *sarc*) which is quite superfluous for viral replication, but which is essential for the maintenance of the malignant state of fibroblasts.

Stehelin, Guntaka, Varmus and Bishop (*J. molec. Biol.*, **101**, 349; 1976) have prepared a specific cDNA_{sarc} probe complementary to the *sarc* gene of the virus. This was accomplished by transcribing complementary DNA of non-defective RSV by its endogenous RNA-directed DNA polymerase activity, followed by molecular hybridisation to the RNA of a transformation-defective deletion mutant. The non-hybridised cDNA, which can be separated on a hydroxyapatite column, represents a uniform transcript of the deleted sequences. Nucleotide sequences homologous to avian cDNA_{sarc} seem to be present in all avian sarcoma viruses, but are absent from avian leukosis viruses, and from sarcoma and leukaemia viruses of mammals. On page 170 of this issue of *Nature*, Stehelin, Varmus, Bishop and Vogt demonstrate that cDNA_{sarc} hybridises with DNA from normal chicken cells.

Previous studies by Neiman, *et al.* (*J. Virol.*, **13**, 837; 1974) indicated that the hybridisation of sarcoma virus RNA to normal chicken DNA was entirely blocked in the presence of excess RNA from transformation-defective

Molecular analysis of the oncogene

from Robin Weiss

viruses, suggesting that sarcoma-specific sequences were not present in the normal chicken genome. The discrepancy between their results and those of Stehelin *et al.* remain to be explained. Thermal denaturation experiments by Stehelin *et al.*, however, show a 4 °C depression in melting temperature (T_m) of cDNA_{sarc} duplexes with normal chicken DNA by comparison with the T_m of duplexes with the provirus of RSV. This indicates substantial mismatching between the viral *sarc* sequences and those endogenous in the chicken cell. Under Neiman's more stringent conditions of hybridisation, such partially homologous sequences might not be detected.

Hybridisation of cDNA_{sarc} to DNA of other avian species reveals a widespread occurrence of *sarc*-like sequences in normal DNA, including that of a primitive ratite bird, the emu. The melting temperatures of the duplexes indicate a divergence of *sarc* sequences roughly in parallel with species divergence, in contrast to a much more rapid divergence of endogenous leukaemia virus gene sequences. Stehelin *et al.* anticipate that the cellular DNA related to cDNA_{sarc} serves some function which accounts for its conservation during avian speciation. The sequences are part of the unique fraction of cellular DNA and could represent either structural or regulatory genes. The *sarc* gene of the virus may have been acquired from a cellular genome, possibly (in view of the melting curves) from a species other than the chicken.

It is not yet clear whether the *sarc*-like sequences in normal avian cells are specifically expressed in normal embryogenesis or following non-viral carcinogenesis, as suggested by Huebner and Todaro when they enunciated the viral oncogene concepts (*Proc. natn. Acad. Sci. U.S.A.*, **64**, 1087; 1969). Bishop and Varmus' laboratory is currently studying this question.

The widespread occurrence of sequences related to cDNA_{sarc} in avian species will doubtless stimulate more intensive studies of *sarc* sequences in

mammalian sarcoma viruses. Scolnick, Parks and colleagues (*J. Virol.*, **12**, 458; 1973; **13**, 1211; 1974) have detected sarcoma-specific sequences in different strains of murine sarcoma virus (MSV). In the Kirsten and Harvey strains, the *sarc* sequences, plus other viral sequences, seem to be derived from a rat host, whereas Moloney strain MSV is related to the mouse only. It will be interesting to find out whether the '*sarc*' sequences of feline and simian sarcoma viruses are related to those of MSV, and, by analogy with the wide phylogenetic representation of *sarc* sequences among avian species, whether any of these sarcoma viruses will be useful in probing *sarc*-specific expression in human tumours.

There is no genetic evidence so far of specific oncogenes in leukaemia viruses of any kind. Viruses that are typically leukaemogenic *in vivo* but can also transform fibroblasts *in vitro*, such as murine Abelson virus and avian MC29 virus, do not seem to carry *sarc* sequences (Scolnick *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 4650; 1975; Graf and Stehelin, personal communication), and neither are *sarc* sequences detectable in the cellular RNA of Abelson-transformed fibroblasts. Thus these viruses may transform cells by a different mechanism or gene product.

If the viral *sarc* sequences do code for a specific protein, it may be possible to identify it by comparing *in vitro* translation products of sarcoma viruses with those of their transformation-defective deletion mutants. Such attempts by several laboratories have not, however, yielded an identifiable protein so far. Of course, the isolation of an 'oncoprotein' coded by the 1,600 or so nucleotides of the '*sarc*' gene is a major goal of oncornavirologists today. How such a protein might interact with the host cell is an open question. Some virologists expect it to be a nuclear protein which would effect regulation of DNA synthesis, whereas others feel it may act elsewhere, by insertion in the cell membrane, for example. In this case the transformation-specific cell surface antigen of virally transformed avian cells is already a candidate. Since a nuclear antigen corresponding to papovavirus T antigen or EBNA of cells transformed by Epstein-Barr virus has not been identified in cells transformed by sarcoma viruses, I currently favour a non-nuclear role. □

Maternal effects on development

from Michael H. C. Snow

DURING the past 50 years the attention of embryologists has been much taken up with induction and activation. For them these terms are descriptive of the intercellular and intracellular processes whereby, presumably as the result of information received (or lost), a tissue or cell is made to modify its course of development. To the bacterial geneticist, however, induction and activation imply the mechanisms involved in the control of gene expression. Because cell differentiation depends on qualitative changes in gene activity, the terms should eventually come to mean the same to both groups. But at present the bacterial geneticist can refer to precise interactions between inducing or activating molecules and the genes themselves, while this is not possible for the embryologist dealing with higher organisms.

The current state of knowledge of these processes in embryonic development is brought into sharp focus by two recent papers. Toivonen *et al.* (*Differentiation*, **5**, 49; 1976) consider inductive interactions between tissues. Primary induction whereby neural tube formation by the ectoderm is triggered by the underlying mesoderm was discovered, in Amphibia, by Spemann and his colleagues in the 1920s. They later presented evidence that an unidentified chemical substance(s) produced by the mesoderm was the effective agent (Bautzmann *et al.*, *Naturwissenschaften*, **20**, 971; 1932). Since then however, the existence of diffusible inductive agents has been the subject of much controversy, partly because of the difficulty experienced in repeating some of the original experiments, and more recently because Saxén and co-workers demonstrated the necessity for cell contact between tissues in the induction of kidney tubule formation (Lehtonen *et al.*, *J. Embryol. exp. Morphol.*, **33**, 187; 1975). Nevertheless, it is now clear that cell contact is not required for the initiation of primary induction and that some diffusible macromolecular factor is involved. The exact nature of the inducer and its mode of action is still obscure.

Most if not all embryos depend for their early development on material deposited in the oocyte cytoplasm, which is subsequently used during cleavage stages. In some cases maternally derived cytoplasmic factors are required for the normal development of much later stages. Deficiencies in the oocyte cytoplasm (for example, those resulting from mutation in the maternal genome) can therefore profoundly

affect the development of the embryo. Several such maternal effect mutations are known in various organisms, such as the grandchildless mutant in *Drosophila pseudoobscura* (Spurway, *J. Genet.*, **49**, 126; 1948), shell coiling in the snail *Limnaea* (Conklin, *Anat. Anz.*, **23**, 577; 1903) the *O* mutant in the axolotl (Briggs and Cassens, *Proc. natn. Acad. Sci. U.S.A.*, **55**, 1103; 1966) and a recent example has been described in the DDK strain of mouse (Wakasugi, *J. Reprod. Fert.*, **33**, 283; 1973).

The interest in maternal effects stems from the observation that abnormal development occurs although the genetic constitution of the individual is normal. The error apparently results from a failure to activate certain genes during embryogenesis.

A particularly exciting example of a maternal effect gene, the *O* gene in the axolotl is the subject of Brothers' report in this issue of *Nature* (page 112). The crucial observations on the *O* gene are as follows. (1) Development is arrested in embryos derived from homozygous *O* females at the onset of gastrulation. At this time much of the embryonic genome becomes active, in particular the genes coding for ribosomal RNA. (2) The product of the normal allele (*O*⁺ factor) can be removed from the oocyte and on injection into mutant eggs can correct the arrest at gastrulation. (3) The *O*⁺ factor is a protein and curiously is most abundant in the oocyte nucleus. What Brothers has shown, by a series of very elegant nuclear transplant experiments, is that the time of action of the *O*⁺ factor is restricted to a very short period in development of a few hours in the mid-blastula stage. If the *O*⁺ factor can be isolated and purified then the way is open to analyse the manner in which at least one inductive agent operates.

In this context it is worth noting some of the other changes found in embryos at the end of cleavage. For instance, a change has been reported in the nature of histone proteins in *Drosophila melanogaster* (Das *et al.*, *J. Cell Biol.*, **23**, 423; 1964), in the snail, *Helix aspersa* (Bloch and Hew, *J. biophys. biochem. Cytol.*, **8**, 69; 1960) and in the mouse (Alfert, *Expl Cell Res.*, Suppl. **6**, 227; 1958). Histones are known to be potent but nonspecific repressors of transcription. Non-histone proteins on the other hand, have been postulated as being involved in de-repression (activation) (Wang and Kostraba in *The Role of RNA in Reproduction and Development*, (edit. by Niu and Segal) 324, North Holland, Amsterdam, 1973).

The onset of gastrulation is generally accompanied by a significant change in cell proliferation rate, and in the mouse this is coincidental with a change in chromosome replication behaviour (Takagi, *Expl Cell Res.*, **86**, 127; 1974, Snow, *Ciba Symp.*, **40**, in the press). Callan has shown that in Amphibia at least, large changes in the duration of the DNA synthesis phase of the cell cycle are likely to be associated with changes in replicon length rather than changes in the linear rate of DNA replication (Callan, *Proc. R. Soc., Lond.*, **B181**, 19; 1972, and in *Molecular Cytogenetics* (edit. by Hamkalo Papaconstantinou, 31, Plenum, 1973). DNA involved in replication is not available for transcription and the restriction in the number of replicating forks in the DNA may be necessary to permit transcription of large amounts of RNA. The *O*⁺ factor could operate in either of these areas, or alternatively could be very much more specific and result perhaps in the activation of a specific gene. After 50 years of probing, a breakthrough on any front would be welcome. □

Role of ribosomal RNA

from Richard Brimacombe

THE flurry of interest in bacterial ribosomal proteins over the past few years has tended rather to obscure the fact that about 60% of the bacterial ribosome consists of RNA, and many workers have tried to relegate the rRNA to a mere structural component. This attitude has arisen partly as a consequence of the dogma that proteins and not nucleic acids are the active

agents in biological systems, but is also a result of the fact that, in contrast to the studies which can be made with individual ribosomal proteins, it is very difficult to devise functionally oriented experiments involving the large rRNA molecules. The idea that rRNA does indeed have an important functional role is of course not new; it was suggested some years ago by Crick and

A NEW study of the behaviour of the tide within the lagoon of Venice, in itself not a revolutionary idea, promises to have far-reaching practical results in the Italian government's choice of the most suitable barrier to prevent flooding in Venice.

A. Goldmann, R. Rabagliati and P. Sguazzero of the IBM Scientific Centre in Venice in an article in the *Rivista Italiana di Geofisica* (2, 119; 1975) have computed the relation between the level of water outside the lagoon and the level at 14 points within the 50 km² lagoon. The data were provided by hourly readings of water level at the 15 stations during 1972 and 1973. Using spectral analysis, the authors first investigated the transfer of energy from the open sea to every lagoon station at each of a large number of frequency bands. This showed that only in three frequency bands—the long-term, the diurnal and the semi-diurnal—was the transfer of energy significant. The authors then calculated the gain or loss in the amplitude of the diurnal and semi-diurnal waves—those in which the astronomical components are predominant—and the time it took waves of these two frequencies to reach each station (the phase).

In 1971 the lagoon was sounded in detail: the soundings revealed that 75% of the lagoon is shallow water or marsh less than 2 m deep, intersected by the deep channels which make up the other 25%. When this

information is combined with the tide data an interesting picture begins to emerge: on deep channels the semi-diurnal waves, although not the diurnal waves, actually increase in amplitude after entering the lagoon (the gain at the Grand Canal station is 6%); in shallow water on the other hand the waves lose amplitude, the semi-diurnal waves losing more amplitude than the diurnal ones. Propagation of waves is slower in shallow

On the lagoon

from Gillian Boucher

water and the waves of the diurnal tide are propagated more slowly than those of the semi-diurnal.

The detailed results, with the advantages of a long period of data collection, the almost contemporary sounding data, and sophisticated statistical techniques, provide a clearer picture of the lagoon than has ever been obtained before. One important use for them will be to show how the lagoon is changing by comparison with past and future results. But the results have already been used in another of the IBM projects, very recently completed: a mathematical model of the entire lagoon. The model, calibrated by the new tide results and other information, can reproduce the behaviour of water at any point and the effects of man-made changes in the lagoon. One

of the first problems the model will be called upon to solve is the effect upon the lagoon of the various types of barrier which could be erected at its three entrances.

One of the scourges of Venice is the tide which, though small by Atlantic standards, frequently causes flooding because the city is so low-lying. To save the city from the salt-water inundations the Italian government has invited tenders for the construction of a sluice at the entrances to the lagoon; companies interested in tendering must do so by June 30 this year. Of the two basic types of barrier, the moveable and the fixed, the moveable (for example Pirelli's project for an inflatable barrier) are clearly superior but very much more expensive. A fixed barrier completely shutting off the lagoon is out of the question—Venice relies on the tide to wash away industrial and domestic pollution, and to prevent circulation of water would be devastating to both humans and buildings. So the choice is between a moveable barrier and some kind of partial fixed barrier which will lessen the quantity of water entering sufficiently to prevent flooding. Inevitably a fixed barrier will also reduce circulation. The IBM model will enable companies and the government to decide whether a satisfactory compromise can be obtained between the needs to damp the tides and to control pollution.

Orgel (*J. molec. Biol.*, **38**, 367 and 380; 1968) that the primitive ribosome consisted primarily of RNA, with the proteins being added later to fulfil more sophisticated functions. More recently, the functional importance of RNA in the "modern" ribosome has been preached by a number of authors, most notably by Kurland (*Ribosomes*, 309, Cold Spring Harbor Monograph, 1974), and the purpose of this article is to review very briefly the present status of rRNA, with regard to both structure and function.

Little progress can be made nowadays in any nucleic acid problem without previous knowledge of the base sequences involved. In the *E. coli* ribosome, the sequence of the 5S RNA component has been known for many years (Brownlee *et al.*, *J. molec. Biol.*, **34**, 379; 1968), and the primary structure of the 16S RNA is now almost fully established (Ehresmann *et al.*, *Nucleic Acids Res.*, **2**, 265; 1975), although there is still a measure of disagreement as to the detailed sequences of some of the oligonucleotide degradation products (Magrum *et al.*, *Nature*, **257**, 423; 1975). Progress has also been made in the sequencing

of the 23S RNA (Branlant *et al.*, *Biochimie*, **57**, 175; 1975). The primary sequences themselves give a strong hint that the RNA is more than just a structural skeleton within the ribosome; the 5S sequence has been strongly conserved throughout evolution (reviewed by Monier, *Ribosomes*, 141, Cold Spring Harbor Monograph, 1974), and comparison of the oligonucleotide catalogues from 16S RNA molecules from different bacteria shows a remarkable degree of homogeneity (Woese *et al.*, *Nature*, **254**, 83; 1975), again indicating that substantial regions of the sequence have been conserved. The obvious inference is that particular oligonucleotide sequences are required to fulfil specific ribosomal functions, and this has tempted several authors to put forward hypotheses involving complementary base-pairing between regions of rRNA and other nucleic acid components of the protein synthesising system (for example, van Knippenberg, *Nucleic Acids Res.*, **2**, 79; 1975). Although these ideas are attractive, it must be borne in mind that in molecules the size of rRNA, any given nucleotide sequence of up to six bases would be expected to appear on a

statistical basis, and solid experimental evidence to support this type of hypothesis is unfortunately very difficult to obtain.

Evidence has however been forthcoming in two cases. The first of these concerns the proposal made by Shine and Dalgarno (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 1342; 1974) that the 3'-terminal sequence of 16S RNA is involved in mRNA recognition. Here, Steitz and Jakes (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 4734; 1975) have been able to isolate a dissociable oligonucleotide complex consisting of the 3'-terminus of the 16S RNA and an RNA fragment from a protein initiator region of R17 phage RNA (see also News and Views, **260**, 12; 1976). The two fragments contained a seven-base complementary sequence. The second case concerns the 5S RNA, where several lines of evidence suggest that binding of tRNA to the 50S ribosome involves a complementary base-pairing between the T ψ CG loop of the latter with 5S RNA (Erdmann *et al.*, *Biochem. biophys. Res. Commun.*, **54**, 942; 1973). Here there is some conflicting evidence, as the relevant G-residues on the 5S RNA

are not exposed (within the ribosome) to attack by kethoxal, a reagent which specifically attacks non-paired guanine bases (Noller and Herr, *J. molec. Biol.*, **90**, 181; 1974). This difficulty can of course be overcome by postulating appropriate conformational changes (Schwarz *et al.*, *Biochem. biophys. Res. Commun.*, **56**, 807; 1974).

Kethoxal has been successfully used as a probe for the accessibility of G-residues on the surface of the 30S ribosomal subunit (Noller, *Biochemistry*, **13**, 4694; 1974), and these studies have also provided information regarding the vexed question of the secondary structure of the rRNA. There is still no proven secondary structure for the 5S RNA, and, while a secondary structure has been proposed for *E. coli* 16S RNA (Ehresmann *et al.*, *Nucleic Acids Res.*, **2**, 265; 1975), there is at present little evidence to support it. Indeed the kethoxal data are to a considerable extent at variance with the proposed structure, and a further complication has recently arisen with the finding that specific regions of the 16S RNA are involved in stable long-range interactions with other parts of the molecule (Mackie and Zimmerman, *J. biol. Chem.*, **250**, 4100; 1975). This suggests that secondary structure model-building based on a simple linear array of hairpin loops may be *a priori* invalid.

Many questions concerning both secondary structure and function of the rRNA will perhaps become clearer with a better understanding of the interactions between RNA and protein within the ribosome. This is one of the most important problems of ribosomology, but again it has not proved an easy one to approach. Most of the available data has been obtained by

Garrett and Zimmermann and their co-workers from synthetic complexes between single ribosomal proteins and RNA (reviewed by Zimmermann, *Ribosomes*, 225, Cold Spring Harbor Monograph, 1974). These complexes are subjected to a mild nuclease digestion, following which the RNA region "protected" by the protein can be identified. The approach is of course limited to a study of those proteins which can individually form specific complexes with RNA, the "RNA binding" proteins, but it has nevertheless provided a great deal of information in a number of cases. The size and complexity of the RNA regions involved varies considerably; at one end of the scale are proteins such as S8 and S15 which are found in association with short regions of 16S RNA of the order of 50 nucleotides long. Similar short regions of 5S RNA have been found corresponding to proteins L18 and L25. At the other end of the scale are proteins S4 and L24, both of which interact with very large sections of 16S or 23S RNA approximately 400 nucleotides in length. How far these "binding sites", in particular the very large ones, reflect the situation within the intact ribosome remains to be seen.

For the examination of RNA-protein interactions within the ribosome itself, especially those involving proteins which are unable to bind individually to RNA, another approach is necessary. One method has been to isolate fragments from the complete ribosome following nuclease digestion, and to analyse those RNA sequences which interact with particular groups of proteins in the fragments (for example, Yuki and Brimacombe, *Eur. J. Biochem.*, **56**, 23; 1975); further development of this approach has however been severely limited by the ease with which the specificity of the RNA-protein interaction is lost. A more promising approach, now being undertaken by a number of laboratories, involves the development of suitable RNA-protein cross-linking techniques. One example has been the cross-linking of proteins to the 3'-terminus of the 16S RNA, by way of the periodate oxidation of the 3'-terminal ribose. The proteins concerned are S1 and S21 (Czernilofsky *et al.*, *FEBS Lett.*, **58**, 281; 1975), which is interesting in view of the foregoing discussion of initiation, since both these proteins are involved in mRNA binding. S1 has also been shown to bind to an RNA fragment from the 3'-terminus of the 16S RNA (Dahlberg and Dahlberg, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2940; 1975).

Cross-linking of protein to RNA within the bulk of the rRNA requires more general methods, but here also a measure of success has been re-

corded, such as the identification of some peptides of protein S4 which are in contact with the 16S RNA (Ehresmann *et al.*, *FEBS Lett.*, **58**, 106; 1975), or the identification of a region of 16S RNA which is in contact with protein S7 (Rinke *et al.*, *Eur. J. Biochem.*, in the press), in both cases after cross-linking induced by ultraviolet irradiation. These techniques clearly need a lot of refinement, but an extension of this type of study, coupled with the development of more sophisticated cross-linking reagents, seems at the moment to hold the most promise for the immediate future. The recent application of various physicochemical techniques to the protein topography problem is very rapidly giving us a firmer knowledge of the spatial distribution of the ribosomal proteins; it will not be too long before we have at least a first approximation as to how the rRNA is threaded through this protein matrix. This would be invaluable in studies of both structure and function.

Models from maps

from S. D. Dover

ANALYSIS of the electron density maps which are the results of an X-ray crystallographic investigation is the last stage in protein structure determination.

In the initial development of the subject maps were analysed by placing markers in regions of density corresponding to an identifiable feature, measuring the marker co-ordinates, and adding to or adjusting a wire model of the structure accordingly. The interpretation of a new part of the map frequently relied on the existing portion of the model. This feedback was facilitated by the Richard's box (*J. molec. Biol.*, **37**, 225; 1968) a construction which enabled an image of the model in a half-silvered mirror to be superimposed on a stack of perspex sheets on which were drawn the electron density contours. Alterations or additions to the model were then seen directly in the context of the map and any unexplained or poorly fitted features were made obvious.

The use of computers as aids in the building of models started with Diamond's automatic procedures to fit a model to coordinates read from the map (*Acta Cryst.*, **21**, 253; 1966). This approach has been extended to the refinement of a model to fit an electron density map itself ("real-space" refinement), rather than guide coordinates (*Acta Cryst.*, **A27**, 436; 1971). Refinement is an essential part of the structure-solving process and Drenth and Steigmann (*Acta Cryst.*, **B31**, 238; 1975) have taken Diamond's method farther by improving the phasing of the



A hundred years ago

WE cannot see the force of some of the arguments with which Captain Moresby supports his plea for annexation [of New Guinea]. His strong attachment to the natives and his desire for their welfare we think mislead him as to how this is to be accomplished. If New Guinea is to be colonised by white men, all previous experience teaches us that the natives will inevitably suffer, will be demoralised, and ultimately extinguished. It is inexpressibly sad to think of such a fate overtaking these gentle and altogether superior natives of New Guinea; but how can it be helped, unless it is resolved to put a stop to the increase in the white portion of the world's population.

from *Nature*, **13**, March 9, 365; 1876.

structure factors. They used the model fitted to a purely experimental electron density map to calculate structure factor phases for a new map. By repetition of this process the model and the phases were cyclically refined together.

Diamond and Levitt (*J. molec. Biol.*, **82**, 371 and 393; 1974) attacked another problem of the refinement of models, that of energy, both bonded and non-bonded, attractive and repulsive. In their work on the structure of lysozyme they alternated energy and real-space refinement finding that the alterations suggested by energy considerations tended to improve the fit to the electron density data.

This use of energy calculations in conjunction with refinement against an experimental map obviated one of the criticisms of those who advocated the building of an actual model and its improvement as the only way of introducing intramolecular and intermolecular interactions. Nevertheless the viewing of a model superimposed on an electron density map has been not only a precursor of refinement but also important itself. Even where methods of refinement have been used to extract as much information as possible from the diffraction data without recourse to model building, a model must be built to examine the biological implications of the structure.

The difficulties of constructing a very large Richard's box, of reading off the coordinates from one, of having either several boxes or being prepared rapidly to interchange models and density maps made the idea of a computer driven visual display system attractive. Levinthal (*Sci. Amer.*, **214**(6) 42; 1966) started drawing, on a visual display, skeletal models of proteins which could be viewed from different angles or caused to rotate. The ability to alter or add to a computer drawn model and the simultaneous display of a three-dimensional density map has since been developed and in a number of laboratories (see for example *Fedn Proc.*, **33**, 2368; 1974 and *Science*, **190**, 1047; 1975).

Given a refinement system and a good catchnet to enable one to examine by eye those regions of the model which appeared to be problematical, the recent work by Greer (*J. molec. Biol.*, **100**, 427; 1976) would imply an ability to analyse completely an electron density map without ever actually looking at it in its entirety. One can envisage a protein structure so large that even with new display technology its map could not be viewed in one picture. The building of a model using the display system would require excessive searching across the boundaries of the displayed region. Preliminary automatic computer model building by Greer's method followed by examina-

tion using a stereoscopic visual display system would then provide a suitable preparation for refinement.

A large structure such as this would not be amenable to the building of a physical model after refinement. Studying the computer model would then also require a stereoscopic display system and techniques for searching through a large three-dimensional structure which can be seen, as it were, through a small window. Such a display system would facilitate comparison of structural results from other laboratories and the teaching of structural principles. It would, however, lack the presence of a good old-fashioned wire model. □

Contraception

from J. P. Hearn

A meeting on Contraceptives of the Future convened by R. V. Short and D. T. Baird was held by The Royal Society on February 18 and 19, 1976. The invited speakers examined the advantages, disadvantages and the problems of distribution of currently available methods for controlling fertility, and discussed new techniques that are being developed.

CONTRACEPTION is here to stay. In the past the natural checks on human populations were a relatively late age at puberty, high neonatal mortality, a long interval between births and a short life expectancy. Man has reversed all of these natural controls in most developed and developing societies. Menstruation now begins at about 11 years of age, neonatal mortality is very low, urbanisation and improvements in nutrition have more than halved the natural lactation-induced interval between births, and average life expectancy has almost doubled.

All these naturally occurring limiting factors were female orientated mechanisms, and since natural selection has always operated in the past to maximise reproductive potential, women may be physiologically ill adapted to spend the greater part of their reproductive lives in the non-pregnant state. As far as possible, new contraceptives should therefore be designed to mimic the physiological state found during periods of natural infertility in women. There are already many methods of contraception available but cultural, political and medical considerations make few of them universally acceptable so that there is a pressing need for new methods to be developed for both women and men. Ideally any new method should be long lasting, cheap, reversible, convenient and free from

Archaean-Proterozoic polar wandering

from Peter J. Smith

THERE is now considerable geological and palaeomagnetic evidence to suggest that there were no large relative movements of plate segments during the Proterozoic. There was almost certainly some internal deformation along mobile zones between rigid cratons, although any relative motion such a process may have involved was evidently too small to be resolved by current palaeomagnetic methods. In any event, the palaeomagnetic data analysed by Piper *et al.* (*Nature*, **245**, 244; 1973) showed that for the period 2,000–800 million years ago the apparent polar wandering paths for reconstructed Africa and North America (the two most widely studied continents) were in close agreement.

But what about the period before 2,000 million years ago? Until recently it would have been difficult to answer this question because appropriate palaeomagnetic data were few and many more of those that were available were assigned unreliable ages. According to Piper (*Earth Planet. Sci. Lett.*, **28**, 470; 1976), however, this situation no longer obtains, at least for all of the period before 2,000 million years. Not only are there now a considerable number of relevant palaeomagnetic poles available but many of them have been well dated, particularly by the Rb-Sr method.

Because igneous activity during the Precambrian was episodic, some time intervals are better represented in the palaeomagnetic record than others. The two best to date are 2,160–2,000 million years and 2,700–2,500 million years. Piper concludes that throughout both of these periods the combined poles from Africa and North America lie on a single apparent polar wandering curve when the two continents are reconstructed on the basis of the younger Precambrian palaeomagnetic data. In short, the two continents apparently acted as a single unit, albeit with some internal deformation undetectable palaeomagnetically, throughout the whole period 2,700–800 million years ago.

the need for continual medical supervision.

Expenditure on the development and application of contraceptives is only 2% of all the funds going to biomedical

research. The current level of funding, an annual \$120 million, has been steadily reduced over the past three years by inflation. Most funding comes from the developed countries, where the lower birth rates now achieved may inhibit governments from future spending on contraceptive research. But research in reproduction may also pay off in the treatment of infertility, which is the other side of the same problem. At present basic research accounts for 60% of the funds, the development and application of contraceptives takes 30%, and the remaining 10% goes into training personnel and into testing for safety. This latest component will have to be increased in the future as ethical and drug administrative regulations have become increasingly stringent. Investment in contraceptive research is essentially long-term, and seldom commercially rewarding. Pharmaceutical companies are unlikely to spend as much on contraceptive research in the future since testing on several animal species, including monkeys, makes it already a great deal more expensive to bring a new contraceptive on to the market than, for example, a new drug for the treatment of heart disease. The problems of regulating human fertility will not be solved in the near future and by the early 1980s the level of expenditure will need to be about \$500 million each year.

Undoubtedly the most effective and acceptable contraceptive available is the combined gestogen-oestrogen pill. Although this method has a few disadvantages and does not suit all women, it will continue to be the most important contraceptive used over the next ten years in the developed and developing countries. The ways in which it is introduced and marketed in developing countries require a great deal more thought, adapting the approach to a wide variety of cultural differences. The pill causes infertility by creating the hormonal state normally found during pregnancy. It may be possible to develop other types of pill to mimic the hormonal states found during lactation or before puberty, the other naturally occurring periods of infertility in women. Condoms are still probably the most widely used method in many countries and although they are not so reliable, their simplicity and lack of any disadvantageous effects makes them the method of choice for many people. Intra-uterine devices and hormonal implants still require a considerable amount of development before they are as effective or as acceptable. Abortion in early pregnancy is clearly effective, but requires medical or paramedical supervision and is not a contraceptive. In some countries abortion has been used widely but to many people it represents an un-

acceptable policy of despair, a consequence of the failure to produce and distribute adequate contraceptive technology.

There have been advances in the field of surgical sterilisation, particularly in the female, that hold promise for a simple, reversible method. Developments in colpotomy, culdoscopy and laparoscopy have minimised the surgical procedures involved and markedly reduced the time required for recovery. Attempts to use removable clips on the oviducts may lead to "temporary sterilisation" as a means for spacing a family rather than an irreversible technique to be applied only when the family is complete. In the male, sterilisation by vasectomy is widely used but as yet reversibility is not generally possible. There has been an interesting trend in that sterilisation is becoming more accepted amongst women and now as many women as men are being sterilised when they have completed their families.

The World Health Organization, in its expanded programmes of research in human reproduction, is supporting research projects aimed at improving most of the currently available methods of fertility control and also at developing new methods. WHO and other organisations, such as the Population Council and the Ford Foundation, are supporting research into more reliable ways of predicting ovulation and so using abstinence during the critical stages of the natural cycle as a method of birth control. This approach is complicated as the human sperm can survive in the female genital tract for up to seven days, but if ovulation could be predicted more precisely such methods may help those who find other methods unacceptable for cultural or religious reasons. Research is also going on to examine naturally occurring plant substances that have been used as contraceptives in the past in some societies.

It seems unlikely that any new method of fertility control that fulfils the requirements of being long lasting, cheap and reversible will be widely available in the next 5-10 years but two new concepts that may develop relatively rapidly are a pill for men and immunological control of fertility in men and women. Tests on small numbers of men have shown that a combined gestogen-androgen pill will suppress spermatogenesis and not interfere with libido, but the variation in response to this treatment is too wide at present and further tests are necessary to ensure its efficacy and to examine any possible toxicity. At present it seems likely that this method will be reversible.

Studies on marmoset monkeys have shown that it is possible to immunise

primates against a fraction of chorionic gonadotropin, a hormone normally produced in the earliest stages of pregnancy which is thought to stimulate the production of progesterone from the ovary. Immunised marmosets will remain infertile for many months, but a great deal more research into possible side effects is necessary before this method can be applied. In particular it is necessary to show that the antibodies do not cross react with endogenous hormones nor have any effects on foetuses as the contraceptive effects of the vaccine wear out. In the meantime research in India has shown that human chorionic gonadotropin can be made antigenic in women and in the United States researchers have made synthetic fractions of chorionic gonadotropin that may, in the long term, enable the production of a non cross-reacting and highly specific vaccine. Further work is in progress to try to make the vaccine reversible. The fact that human hormones can be made antigenic in the human opens up the immunological possibilities of fertility control, but any vaccines that are developed against hormones or enzymes involved in the development or capacitation of human gametes will need to act specifically and not upset other components of the system.

If we accept that from now on we shall be dependent on artificial forms of contraception then tomorrow's contraceptives must be chosen with great care, as they will have a considerable impact on our general health and social well being. □

Sickle cell disease

by Stuart J. Edelstein

A symposium on Molecular and Cellular Aspects of Sickle Cell Disease was held in Dallas on December 10, 1975, under the sponsorship of the Sickle Cell Disease Branch of the National Heart and Lung Institute and the University of Texas Health Science Center.

Sickle cell disease is a genetic abnormality that is understood in many details: a base change A→C in the DNA of the β -globin gene leads to the Glu→Val change at the β -6 positions of haemoglobin. The mutant form, designated haemoglobin S, has diminished solubility in the deoxygenated state and self-associates into the long, rigid fibres that distort the red cells into the characteristic sickle shape and are responsible for the various symptoms of the disease. An earlier hope that cyanate treatment would provide a suitable therapy for controlling sickling crises has faded

and the search for an anti-sickling agent continues. Such an agent could be found by the trial-and-error screening of many compounds or by design based on the molecular details of fibre formation which are now emerging and which were summarised at the Dallas symposium.

The day began with B. C. Wishner (Johns Hopkins University, Baltimore) reporting on the structure of a crystalline form of haemoglobin S that may provide clues to the contacts between molecules in the fibres. In the crystals, molecules are arranged in paired strands, with adjacent strands within each pair staggered by half a molecule and rotated by half a turn. An intriguing feature of this crystal structure is that the β -6 position lies at an intermolecular contact between the strands within each pair, and several other sites implicated in the sickling process are also at or near contacts. If the axis of the strands in the crystals corresponds to the fibre axis *in vivo*, the orientation of the molecules in the crystals also satisfies critical optical dichroism data. Thus the juxtaposition of molecules in the crystals could bear a close resemblance to the arrangements in the fibres (with minor adjustments for converting the planar alignment of the crystals to the helical alignment of the fibres).

The possibility of a close relationship between the crystals and the fibres was strengthened by the presentation of S. J. Edelstein (Cornell University, Ithaca) on the structure of the fibres determined by electron microscopy, in collaboration with R. Josephs (Weizmann Institute of Science, Rehovot). The fibres were prepared by lysis of sickled cells directly with solutions of negative stain, revealing eight-stranded fibres with adjacent strands staggered by half a molecule as the predominant form in all 10 individuals with sickle cell disease examined. The structure of the fibres was deduced from optical diffraction and Fourier transform analysis of the electron micrographs and is consistent with an assembly of four pairs of strands in the general arrangement found in the crystals studied by Wishner *et al.* Uncertainties remain, including whether adjacent pairs of strands would be parallel or antiparallel and why the eight-stranded, staggered-strand structure was not detected in earlier studies by Finch *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 718; 1973).

On the basis of solubility studies of artificial hybrid haemoglobin constructed of β -S and various α -chain mutant forms, R. Benesch (Columbia University, New York) reported five new α -chain positions apparently involved in sickling. The two positions

Light in dielectrics

from a Correspondent

For the past sixty years there has been disagreement about the ratio of momentum to energy carried by electromagnetic waves in dielectrics. Abraham said $1/nc$; Minkowski said n/c , where c is the speed of light *in vacuo* and n the refractive index, so that c/n is the speed of light in the medium. The question relates to the correct choice and interpretation of the energy-momentum tensor. Subsequent contributors though relatively few have been exceptionally distinguished. This is doubly surprising. First, electromagnetic theory leaves no genuine scope for controversy; second, when the fog began to clear around 1973, the question was found to turn on a clear and detailed description, on the molecular level, of what precisely is measured in any given experiment. In other words the apparent simplicity of the problem as originally formulated proved deceptive.

Continuing the early and especially elegant discussion by Ginzburg (*Sov. Phys. (Uspekhi)* **16**, 434; 1973) and Gordon (*Phys. Rev.*, **A8**, 14; 1973), further steps towards clarifying the issue have now been published by Sir Rudolf Peierls (*Proc. R. Soc. Lond.*, **A347**, 475; 1976). Central to the issue is the distinction between the purely electromagnetic momentum (which by itself would lead to Abraham's result), and the mechanical momentum induced in the molecules of the medium. In an infinite amorphous

(fluid) medium a broad enough light pulse leaves no mechanical disturbance in its wake; but on reflection from an interface it deposits mechanical stresses and strains just upstream of the interface, and these must be considered carefully in accounting for any observations. Professor Peierls has found that to leading order in (n^2-1) , the operative result is the mean of Abraham's and Minkowski's. To higher order in (n^2-1) , there may be need to reflect further on this result, which, (as Peierls himself discusses), is *prima facie* in conflict with some arguments in the classic text by Landau and Lifshitz (*Electrodynamics of continuous media*, Pergamon Press, 1960). For narrow beams (with width to length ratio less than the ratio between the speed of sound and light), there are other effects, some of experimental importance considered already by Gordon (*op. cit.*), and another fascinating one found by Professor Peierls, reminiscent of Cerenkov radiation, of sound generation by the light pulse.

One thing that will surely be needed soon is a systematic textbook-level discussion explaining *ab initio* what results are applicable in what circumstances. For instance, a very recent low-frequency experiment on a solid dielectric (Walker *et al.*, *Can. J. Phys.*, **53**, 2577; 1975) is accounted for by Abraham's result alone, while in many quantum-mechanical applications Minkowski's result, alone, can be used; the "momentum" implied by the latter is then analogous to the so-called "crystal-momentum" of phonons.

with the strongest influence on sickling, α 47 and α 54, were among the α -chain locations predicted to lie at contacts from the model building based on the electron microscopic results noted above. Thus several lines of investigation are converging on the structural details of the fibres, and a definitive description may soon emerge. While such information should help in the design of anti-sickling agents, there are also likely to be many physical-chemical subtleties in fibre assembly yet to be appreciated. One such subtlety emerged from the presentation of A. N. Schechter (National Institutes of Health, Bethesda) concerning a synthetic peptide corresponding to the first 13 residues of the β -S chain. The peptide binds to an antibody elicited in response to haemoglobin S, but does not itself influence fibre formation. Conformational differences between normal haemoglobin and S were also reported by C. Ho (University of Pittsburgh) on the basis of nuclear magnetic resonance studies. Therefore

some aspects of conformation may be critical, along with other possible factors related to intermediate structures and rate limiting steps in fibre assembly.

The 11 other presentations dealt principally with aspects of fibre assembly using various techniques to monitor assembly, including an elegant application of water proton relaxation rates reported by M. R. Waterman (University of Texas, Dallas). Various stages in the assembly process were described on the basis of viscosity by H. F. Bunn (Harvard Medical School, Boston) and ultracentrifugation by R. W. Briehl (Albert Einstein College of Medicine, New York). W. A. Eaton (National Institutes of Health, Bethesda) presented a particularly cogent analysis of the delay time, t_d , for fibre formation, in relation to the "supersaturation ratio", S , raised to a power, n , equivalent to the order of the reaction:

$$1/t_d = \gamma S^n$$

where S is defined as the total concentration of haemoglobin S divided by the

minimum concentration for fibre formation and γ is a kinetic coefficient. Since red blood cells remain for about 1 s in capillaries, the delay time is the critical factor in whether or not a cell sickles and since the order is extremely high (about 40th), small changes in the parameters contributing to S can greatly alter the delay time. For example, the analysis indicates that the delay time in sickle cell trait is about 25,000 times longer than for sickle cell disease. Thus individuals with the trait are essentially free of symptoms and even a small increase in the delay time, as produced by some therapeutic agent, could greatly ameliorate the clinical course of the disease.

The sickling process is also complicated by factors related to the cell membrane. This point was emphasised in the presentation of M. J. Messer (University of California, San Francisco) who discussed experiments on cell sickling monitored by filterability, light microscopy and electron microscopy. When sickling is initiated by rapid deoxygenation, cell rigidity is detected before intracellular fibres or shape changes in the cells appear.

In summary, while considerable progress is being made in sickle cell disease, the complexities of a highly co-operative anisotropic assembly reaction in the confines of a cell membrane are likely to hold many surprises. The latest findings as reported at the Dallas symposium should soon be available to interested readers in detail, since the organisers promise prompt publication of the proceedings. □

Calculating radiation risks

from a Correspondent

A meeting organised by the Society for Radiological Protection was held in London on February 3.

WHAT risks are you prepared to accept and what risks does your society seem prepared to accept? These were among the questions tackled by W. D. Rowe (US Environmental Protection Agency). The problem of assessing the biological effects of exposing large populations to small amounts of ionising radiation is currently a live issue in radiological protection circles. The man rem is the unit for the quantity 'collective dose equivalent' used in discussions of this question and the theme of the Society's meeting was the application of the man rem concept. Rowe pointed out that a central issue was the association of man rems with risk. The risks

were to be balanced against the benefit which the population derived from the practices giving rise to the exposure. There were different types of risk, those arising from the planned releases of radioactive materials and those resulting from accidental situations. Rowe went on to analyse the factors important in the evaluation of risks and accepted that value judgments were an essential part of this procedure. The Environmental Protection Agency had, for example, used collective dose for regulatory purposes. They had set standards and determined the cost of carrying these out by using what Rowe called "operational value judgments" to determine how much money should be spent on "health effect avoidance". He felt that such value judgments were useful in this context for although subjective they did lead to decisions and action. They could be challenged and discussed and had been found to be helpful for that reason.

Any consideration of collective dose must deal with the significance of the large numbers of man rems arising from very small doses to very large numbers of people. P. M. Bryant (National Radiological Protection Board) had some forthright suggestions to offer. She felt that as we were all exposed to natural background radiation and as its level varied, for example, with geographical location, for the purpose of determining collective dose it was not reasonable to integrate doses below a level which fell well within the variation of natural background. She considered that it should be possible to define processes giving rise to exposures of the public in terms of "practices" which were of such a nature that significant numbers of individuals exposed in the overlapping fringe areas of several practices did not unwittingly receive more than the standard deviation of the background, that is, 10 millirems per year. A practice so defined might be, for example, all the long lived nuclides discharged during the 30 years of the life of a nuclear power plant. Bryant thought that if a reduction in the 10 millirem was made by one order of magnitude to allow for the number of practices going on at any one time and by another order of magnitude to allow for considerations of the future, then the resulting 0.1 mrem per year could be used as a cut-off. Below this the doses would be deemed to be insignificant and would not be used in any integration to obtain collective dose equivalent where such integration is intended for making choices between one practice and another. It seemed to her that this cut-off level would assist in realistic cost/benefit analysis although she admitted that if her concept of "collective dose from a practice" were

used the definition of the practice might sometimes present difficulties.

During his discussion of two methods of considering radioactive releases arising from the operation of nuclear-powered electricity generating stations, R. H. Clarke (CEGB Berkeley Nuclear Laboratories) commented that the Canadians neglected doses below 1% of the dose at the site boundary and the Americans also employed a cut-off by integrating only the doses received within 50 miles of the site. Releases could be considered in terms of the doses given to a hypothetical critical group of the public located at the site boundary close to the release point. If the doses to this group were kept within the limits recommended by international authorities, then it followed that the doses received by the rest of the population would also be considered acceptable. This in fact was the approach used at present by the Central Electricity Board, but Clarke pointed out that it was possible for the doses to the critical group to remain within the arbitrary limits, yet, by varying stack heights, the distance from the release point to the site boundary and the distribution of the population around the site, the collective dose equivalent from similar releases of xenon-133 could vary by two orders of magnitude. This indicated a weakness of the critical group approach and in future although the doses to the most exposed individuals would remain important, some kind of limitation on the population collective dose around the site might be a more realistic parameter for the design engineer to aim at.

R. H. Mole (MRC Radiobiology Unit, Harwell) speaking in his private capacity, said it was important to distinguish between the validity of using man rems for thinking about exposures and for adding units of risk, and the validity of converting man rems into cases of detriment or hurt in some sense. Alternative hypotheses concerning the slope of the dose response relationship had to be considered. He was personally inclined to accept the linear hypothesis and was therefore critical of those who proposed a cut-off. It seemed to him that there was no adequate scientific reason for rejecting linearity and he suspected that there must be some "cultural" reason for distrusting it. Perhaps people were uneasy about calculating risks on the basis of a hypothesis which could not be demonstrated to be true.

The meeting was concluded by Mole pleading for those who published estimates of collective dose to set out every step of their calculations and in particular to state the range of uncertainty of the final number. A salutary exercise, no doubt! □

review article

Possible role for cyclic nucleotides and phosphorylated membrane proteins in postsynaptic actions of neurotransmitters[†]

Paul Greengard*

The postsynaptic actions of some neurotransmitters may be mediated through cyclic nucleotides and cyclic nucleotide-dependent phosphorylation of specific membrane proteins in postsynaptic cells. In addition to providing a molecular basis for the actions of several neurotransmitters and of certain drugs affecting behaviour, the model suggests a mechanism by which neurotransmitter signals may be converted into electrophysiological responses in postsynaptic cells.

THE pioneer work of Sutherland, Rall and their colleagues firmly established that cyclic AMP acts as the intracellular mediator for the regulatory actions of many hormones on a variety of non-neuronal tissues¹. There is more recent evidence that cyclic AMP also has an important role in the nervous system, where it seems to participate in certain types of communication between nerve cells. Cyclic AMP is implicated in at least three discrete processes in the nervous system, namely, mediation of the postsynaptic actions of certain neurotransmitters, regulation of the biosynthesis of certain neurotransmitters, and functioning of microtubules. Other evidence suggests that cyclic GMP is also involved in some types of synaptic transmission. This article summarises some of the evidence for a role of cyclic AMP and cyclic GMP as mediators of the postsynaptic actions of neurotransmitters and examines a possible mechanism for their action—regulation of the phosphorylation of specific proteins present in the postsynaptic membrane.

Cyclic nucleotides in synaptic transmission

Neurotransmitter molecules released from the terminals of the presynaptic axon induce a change in the membrane potential of the postsynaptic neurone by interacting with specific receptors on that neurone. This electrical change is referred to as the postsynaptic potential. How the activation of a postsynaptic receptor is transduced into the postsynaptic potential is still unknown; but it now seems that, in some instances, cyclic nucleotides are involved. Some of the experimental criteria that have been examined and found to support this view are presented in Table 1. Some of the neuronal systems for which a number of these criteria have been fulfilled are reviewed elsewhere².

One preparation that has been used to study the possible role of cyclic nucleotides in neuronal function is the mammalian superior cervical sympathetic ganglion. A diagram of the principal synaptic connections and the neurotransmitters believed³⁻⁸ to occur within the superior cervical ganglion is

included in Fig. 1, along with the postulated site of involvement of cyclic AMP and cyclic GMP in the production of the postsynaptic potentials. The postganglionic neurone in this ganglion receives three types of innervation. In the principal pathway innervating the ganglion, acetylcholine released from preganglionic terminals activates nicotinic cholinergic receptors on the surface of the postganglionic neurone, leading to the generation of a fast excitatory postsynaptic potential (f-e.p.s.p.). When several of these f-e.p.s.p.s summate, an action potential is generated and is propagated down the axon of the postganglionic neurone. There is evidence that generation of the f-e.p.s.p. does not involve a cyclic nucleotide. Indeed, since the f-e.p.s.p. is very rapid (of the order of a few ms), it seems *a priori* unlikely that its generation and termination would involve a series of enzymatic reactions of the type that seem to be associated with cyclic nucleotide-mediated processes.

There are two slow postsynaptic potentials which modulate the excitability of the postganglionic neurones: a prolonged hyperpolarisation or slow inhibitory postsynaptic potential (s-i.p.s.p.), and a prolonged depolarisation or slow excitatory postsynaptic potential (s-e.p.s.p.). In contrast to the generation of the f-e.p.s.p., the generation of these modulatory slow postsynaptic potentials does seem to involve cyclic nucleotides (Fig. 1). In one of the modulatory pathways, acetylcholine (ACh) released from preganglionic fibres activates a dopaminergic interneurone leading to the release of dopamine; the released dopamine activates a dopamine receptor on the surface of the postganglionic neurone leading, through a process which seems to involve cyclic AMP, to the generation of the s-i.p.s.p. In the other modulatory pathway, ACh released from preganglionic terminals activates muscarinic cholinergic receptors on the surface of the postganglionic neurone leading, through a process which seems to involve cyclic GMP, to the generation of the s-e.p.s.p.

Almost all of the criteria listed in Table 1 for mediation by a cyclic nucleotide of a postsynaptic potential have been fulfilled for the generation by cyclic AMP of the s-i.p.s.p. in the superior cervical ganglion. To cite just a few examples: (1) electrical stimulation of the preganglionic cholinergic fibres innervating the ganglion⁹, or application of exogenous dopamine to ganglion slices¹⁰, causes an increase in the cyclic AMP content of the ganglion (cytochemical studies indicate that this increase

*Address: Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510.

[†]Adapted from a lecture presented at the Sir Henry Dale Centennial Symposium in Cambridge, UK, September 18, 1975.

Table 1 Some criteria for mediation by a cyclic nucleotide of a postsynaptic potential*

- a Synaptic activation and cyclic nucleotide levels**
- (1) Electrical stimulation of the presynaptic input should increase tissue levels of the cyclic nucleotide.
 - (2) This increase in cyclic nucleotide level should be antagonised by pharmacological agents that block the postsynaptic potential.
 - (3) The increase should not occur when transmitter release is prevented with low Ca^{2+} /high Mg^{2+} .
 - (4) The increase should not occur when the postsynaptic cells are antidromically activated.
- b Neurotransmitters and cyclic nucleotide levels**
- (1) Levels of the cyclic nucleotide should increase when the intact tissue or blocks or slices of the tissue are exposed to the neurotransmitter thought to be responsible for the postsynaptic potential of interest.
 - (2) This increase should be antagonised by agents that block the neurotransmitter-induced changes of postsynaptic potential.
- c Neurotransmitters and adenylate (or guanylate) cyclase**
- (1) A neurotransmitter-sensitive adenylate (or guanylate) cyclase should be demonstrable in cell-free preparations of the tissue.
 - (2) Activation by the neurotransmitter of this neurotransmitter-sensitive adenylate (or guanylate) cyclase should be blocked by the same antagonists as in (a2) and (b2).
- d Cytochemistry**
- (1) Cytochemical techniques should demonstrate that cyclic nucleotide levels increase in the appropriate postsynaptic cells in response to synaptic activation.
 - (2) Similarly, cytochemical techniques should demonstrate that cyclic nucleotide levels increase in the appropriate postsynaptic cells in response to the appropriate neurotransmitter.
- e Phosphodiesterase inhibitors**
- (1) Phosphodiesterase inhibitors should further increase the elevated levels of cyclic nucleotides achieved by activating the synaptic pathway, or by applying the putative neurotransmitter.
 - (2) Phosphodiesterase inhibitors should potentiate the effects on the postsynaptic potential that follow activation of the synaptic pathway, or application of the putative neurotransmitter.
- f Application of cyclic nucleotides**
- (1) The cyclic nucleotide should mimic the physiological effects of activating the synaptic pathway and of applying the putative neurotransmitter.

*Taken from Beam and Greengard².

in cyclic AMP occurs primarily or exclusively in the postganglionic neurone¹¹); (2) a dopamine-sensitive adenylate cyclase is present in homogenates of the ganglion¹⁰; (3) exogenous cyclic AMP, applied to the ganglion, causes hyperpolarisation of the postganglionic neurones, thus mimicking the hyperpolarisation seen on either preganglionic stimulation (the s-i.p.s.p.) or application of dopamine¹²; (4) phosphodiesterase inhibitors, applied to the ganglion, greatly increase the elevation of cyclic AMP seen on preganglionic stimulation⁸ or application of dopamine¹³, and also greatly increase the size and duration, both of the s-i.p.s.p.¹² and of the dopamine-induced hyperpolarisation¹²; (5) various adrenergic and cholinergic agents affect the s-i.p.s.p.^{3,5,6,12,14}, as well as the increase in cyclic AMP which follows preganglionic stimulation⁸, in a fashion predicted by the scheme shown in Fig. 1. Analogous data have been obtained for cyclic GMP in the generation of the s-e.p.s.p.^{11-13,15}.

These studies indicate that cyclic AMP mediates dopaminergic transmission, and cyclic GMP mediates muscarinic cholinergic transmission, in the superior cervical ganglion. They also suggest that the dopamine receptor of the postganglionic neurone is a dopamine-sensitive adenylate cyclase, the activation of which leads to an increase in cyclic AMP. Finally, cyclic AMP and cyclic GMP, through generation of the s-i.p.s.p. and s-e.p.s.p., respectively, seem to regulate the membrane potential of the postganglionic neurone, and thereby modulate nicotinic cholinergic transmission through the ganglion.

Extensive electrophysiological, pharmacological, and cytochemical studies by Siggins, Hofer and Bloom, have provided good evidence that cyclic AMP mediates transmission at the noradrenergic synapse on the cerebellar Purkinje cell¹⁶⁻¹⁹. Stimulation of the noradrenergic fibres to the Purkinje cell reduces its firing rate, an effect mimicked both by noradrenaline and by cyclic AMP applied iontophoretically. The inhibitory effects of stimulating the noradrenergic fibres, or of applying noradrenaline or cyclic AMP, are markedly potentiated by phosphodiesterase inhibitors. Moreover, the inhibitory effects are associated, in each case, with hyperpolarisation of the Purkinje cell and a decrease in membrane conductance. These results indicate that the inhibitory effects of activity in this noradrenergic pathway are mediated through cyclic AMP.

Biochemical characterisation of dopamine receptors in mammalian brain

The studies of the superior cervical ganglion outlined above suggested a possible approach to understanding the molecular nature of dopamine receptors in the brain. Within recent years there has been, for several reasons, a great deal of interest in dopamine receptors in the central nervous system (CNS). There is increasing evidence for dopaminergic transmission within the brain²⁰⁻³⁰ and aberrations of dopaminergic transmission may have a major role in the aetiology of some diseases of the CNS. For instance, hypoactivity of dopaminergic pathways to the caudate nucleus of the brain seems to be at least partly responsible for the symptoms of Parkinson's disease²¹. Conversely, hyperactivity of dopaminergic pathways to the limbic region of the brain may contribute to the symptoms of schizophrenia³¹⁻³⁴. In addition, the parkinsonian side effects frequently seen in patients given antischizophrenic drugs, and possibly the therapeutic effects of these drugs as well, are largely attributable to their ability to block dopamine receptors in the brain^{20,23,29}. Since the dopamine receptor in the superior cervical ganglion seemed to be the dopamine-binding portion of a dopamine-sensitive adenylate cyclase¹⁰, and since a dopamine-sensitive adenylate cyclase was also found in retina³⁵, a search for such an enzyme was undertaken in the caudate nucleus of the brain, a region rich in dopamine receptors.

An adenylate cyclase, activated by low concentrations of dopamine, was found in homogenates of the caudate nucleus³⁶. The enzyme was also activated by low concentrations of apomorphine, a known dopamine-receptor agonist. In contrast, isoproterenol, an effective agonist at β -adrenergic receptors, but not at dopamine receptors, failed, even in high concentrations, to stimulate the enzyme, indicating the specificity of the enzyme activation. Furthermore, the activation of the enzyme by dopamine was strongly inhibited by two classes of antischizophrenic compounds known to be dopamine-receptor antagonists. In contrast, several other pharmacological substances, with no antischizophrenic or dopamine-receptor antagonist properties, failed to block the dopamine activation of the enzyme. The results of this study indicated that the dopamine receptor in mammalian brain (as in the superior cervical ganglion) might be the dopamine-binding portion of a dopamine-sensitive adenylate cyclase, suggested that activation of this enzyme could account for the action of some antiparkinson drugs, and suggested a molecular basis for the actions of antischizophrenic drugs.

Subsequent investigations in several laboratories have strengthened and extended these conclusions³⁷⁻⁴⁶. An adenylate cyclase has been found in the limbic region of the brain^{37,38,43} with characteristics virtually identical to those of the caudate enzyme. Moreover, in studies using a vast array of known agonists and antagonists of the dopamine receptor, the ability of substances to act as activators or inhibitors of the dopamine-sensitive adenylate cyclase system has shown, with few exceptions, a remarkable parallel with their ability to act as agonists or antagonists of the dopamine receptor³⁷⁻⁴⁶.

As an illustration of such studies, Fig. 2 shows the effect of

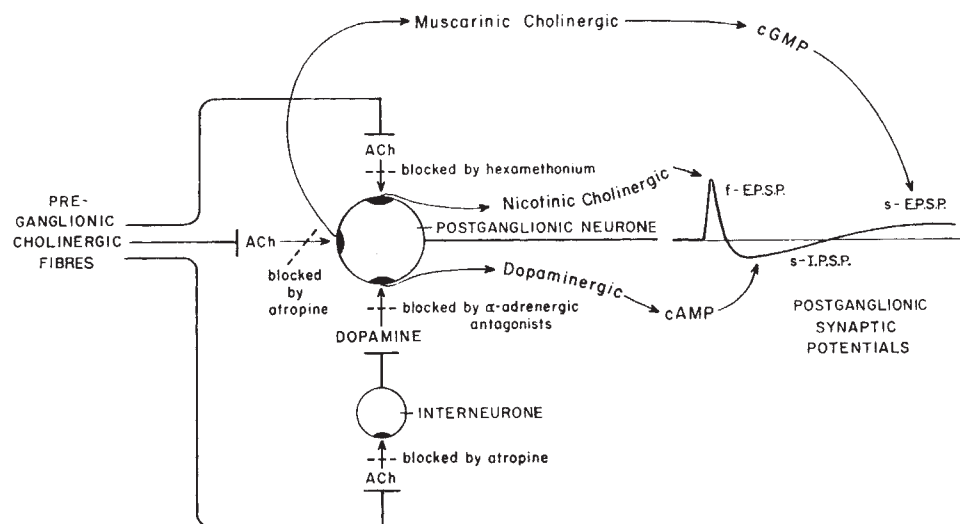


Fig. 1 A schematic diagram of the principal synaptic connections in the mammalian superior cervical ganglion, and the postulated role of cyclic nucleotides in the genesis of the postganglionic synaptic potentials. The diagram shows the relationship between the various neuronal elements; the neurotransmitters released at the various synapses; the sensitivity of the synaptic receptors to different classes of specific antagonists; the electrical signs that accompany activation of the various postganglionic receptors following preganglionic stimulation; and the postulated involvement of cyclic nucleotides in the production of the electrophysiological responses. Abbreviations used: ACh, acetylcholine; f-e.p.s.p., fast excitatory postsynaptic potential; s-i.p.s.p., slow inhibitory postsynaptic potential; s-e.p.s.p., slow excitatory postsynaptic potential. (Based on studies reviewed by Eccles and Libet³, Volle⁴, Libet⁵, Greengard and McAfee⁶, and Greengard and Keibian⁷. Modified from Kalix *et al.*⁸.)

dopamine and of fluphenazine (a potent antischizophrenic drug and dopamine-receptor antagonist) on the adenylate cyclase activity of a subcellular fraction, rich in synaptic membranes, from the rat caudate nucleus. Dopamine caused a half-maximal increase in enzyme activity at about 4×10^{-6} M, which was competitively inhibited by fluphenazine. Moreover, fluphenazine was extremely potent as an inhibitor of the enzyme, with an inhibition constant (K_i) of 8×10^{-9} M (ref. 46). The inhibition of the enzyme by fluphenazine and related compounds occurred at concentrations two to three orders of

magnitude less than those at which most other effects of these substances have been found.

These studies support the conclusion that the dopamine receptor, in at least some regions of the brain, as in the superior cervical ganglion, is part of a dopamine-sensitive adenylate cyclase. A corollary of this conclusion is that cyclic AMP mediates synaptic transmission at such dopaminergic synapses.

Finally, these studies indicate that inhibition by the antischizophrenic drugs of dopamine-sensitive adenylate cyclase in the caudate nucleus may represent the molecular basis for the

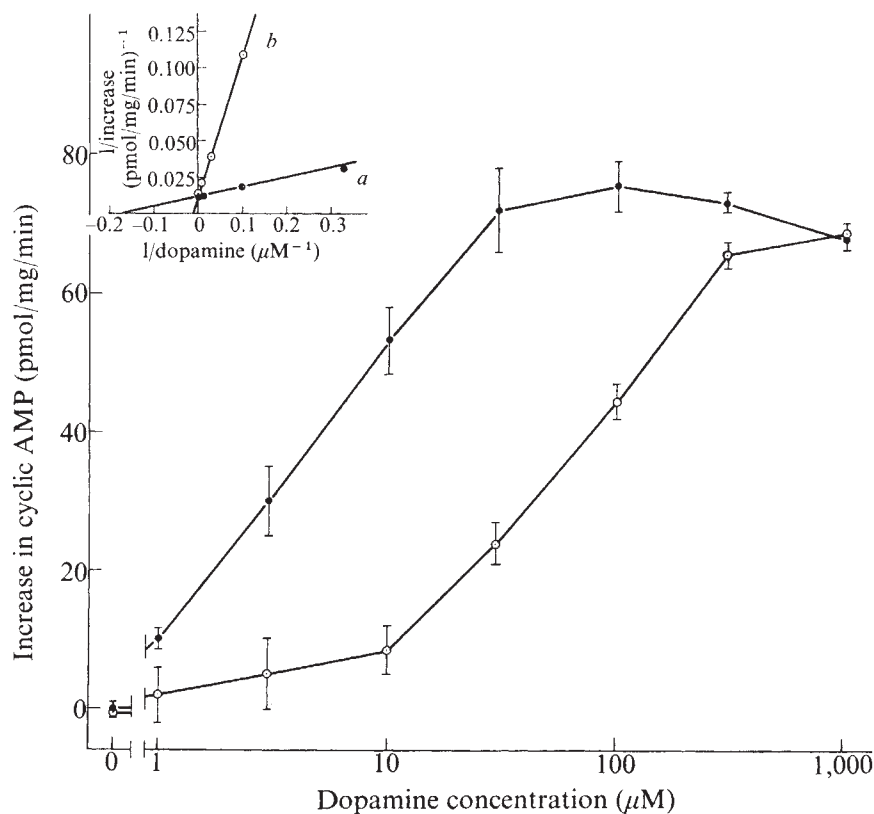


Fig. 2 Effect of various concentrations of dopamine, in the absence (●) or presence (○) of 1×10^{-7} M fluphenazine, on adenylate cyclase activity in a particulate fraction of rat caudate nucleus rich in synaptic membranes. In the absence of added dopamine or fluphenazine, 48.1 pmol of cyclic AMP were formed per mg wet weight of tissue per min; in the presence of 0.1 μ M fluphenazine, 47.9 pmol of cyclic AMP were formed. The increase in cyclic AMP above basal level (that is, the level in the absence of dopamine or fluphenazine) is plotted as a function of dopamine concentration. Inset: double-reciprocal plot of cyclic AMP increase as a function of dopamine concentration from 3 to 300 μ M. *a*, Control; *b*, 1×10^{-7} M fluphenazine. (Taken from Clement-Cormier *et al.*⁴⁶)

parkinsonian side effects of these compounds, and suggest that a similar inhibitory action on the enzyme in the limbic region may be responsible for the therapeutic effects of these drugs.

The evidence that a cyclic nucleotide mediates synaptic transmission is perhaps strongest in the case of dopaminergic synapses, due in part to the large variety of known agonists and antagonists of the dopamine receptor available for study. Nevertheless, cyclic AMP has been implicated in the post-synaptic response to several other putative neurotransmitters, including noradrenaline (acting at β -adrenergic receptors), 5-hydroxytryptamine (5-HT), octopamine, and histamine⁴⁷⁻⁵³. Moreover, the actions of several drugs that affect behaviour, including LSD^{54,55}, morphine⁵⁶⁻⁵⁸, and the antischizophrenic drugs discussed above, may be attributable to actions on specific neurotransmitter-sensitive adenylate cyclases. Finally, studies of the superior cervical ganglion^{11-13,15} and of brain⁵⁹⁻⁶¹ suggest, in the case of one neurotransmitter, namely ACh (acting at muscarinic cholinergic synapses), that cyclic GMP may act as a mediator of synaptic transmission. These various results raise the question of the nature of the receptors for the cyclic nucleotides in the postsynaptic cells.

Phosphorylated proteins in synaptic membranes

The studies summarised in this section have been carried out within the framework of the hypothesis⁶² that the diverse effects of cyclic AMP in various tissues are mediated through regulation of the activity of a family of enzymes known as protein kinases. This class of enzymes catalyses the phosphorylation of substrate proteins by ATP. The protein kinase hypothesis was proposed after the discovery of cyclic AMP-dependent protein kinases, first in muscle⁶³, and then in liver⁶⁴ and brain⁶⁵ and explains how cyclic AMP, a small molecule, may exert the large variety of biological effects attributable to it. According to this hypothesis, cyclic AMP need only regulate the activity of a single class of enzymes, the protein kinases; the specificity of the cyclic AMP action is then accounted for in

terms of the specificity of the protein kinases and, particularly, of the substrate proteins which are phosphorylated by these protein kinases, in the various tissues.

The application of the protein kinase hypothesis to synaptic transmission in the nervous system is illustrated in Fig. 3. Three distinct roles of cyclic AMP and protein phosphorylation in neuronal function, for which there is now evidence, are illustrated. Two of these roles fall outside the main theme of this presentation, and will be described only briefly.

First, cyclic AMP-dependent protein kinase seems to be capable of regulating the biosynthesis of neurotransmitters in presynaptic terminals. Cyclic AMP has been shown to activate tyrosine hydroxylase⁶⁶⁻⁶⁹, the enzyme that controls the rate of biosynthesis of the catecholamines dopamine and noradrenaline. The activation by cyclic AMP of tyrosine hydroxylase is almost certainly mediated through a protein kinase, since the activation by the cyclic nucleotide (1) requires ATP and Mg^{2+} (refs 70-73), (2) is mimicked by a purified preparation of protein kinase⁷⁰, and (3) is abolished by various specific and nonspecific inhibitors of brain protein kinase⁷⁰. Future studies may demonstrate that the rate of biosynthesis of neurotransmitters other than the catecholamines is also subject to regulation by cyclic AMP-dependent protein kinases.

Second, cyclic AMP and protein kinase in nervous tissue may influence the microtubule system. The possibility that cyclic AMP-dependent protein phosphorylation affects microtubule function was first suggested by Goodman *et al.*⁷⁴. Recently, evidence has been obtained⁷⁵ that highly purified microtubules from chick brain contain an intrinsic cyclic AMP-dependent protein kinase which catalyses the stoichiometric phosphorylation of a substrate protein (with an apparent molecular weight of 300,000) that is also an integral component of the microtubule system. Experiments are in progress, using *in vitro* systems, to determine whether the cyclic AMP-dependent phosphorylation of this high molecular weight protein affects some parameter of microtubule function, such

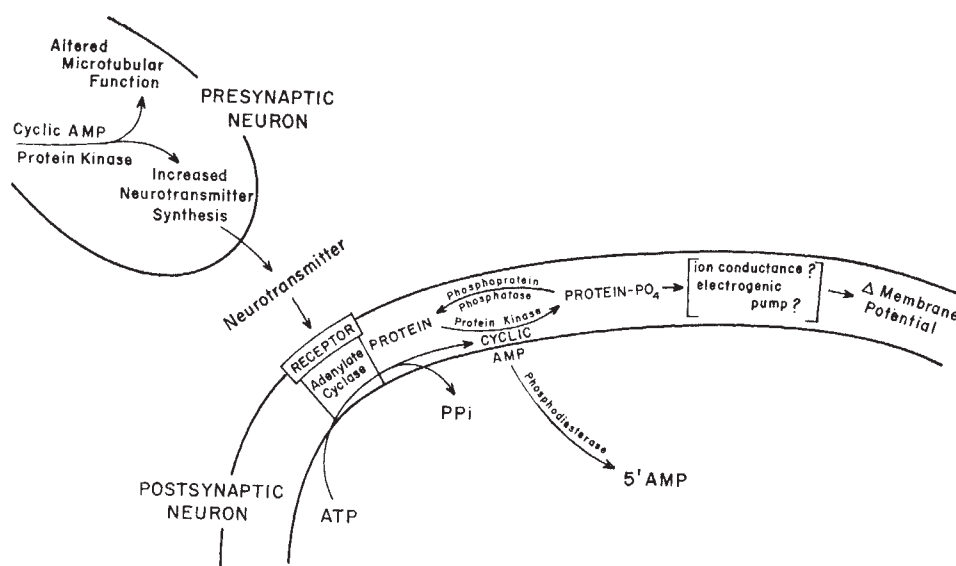


Fig. 3 Proposed roles for cyclic AMP and protein phosphorylation in neuronal function include regulation of neurotransmitter synthesis in presynaptic terminals (a), regulation of microtubular function (b) (indicated, purely for convenience, as occurring in the presynaptic axon; microtubules occur in dendrites, soma and axon of neurones), and generation of postsynaptic potentials (c). The sequence of events by which neurotransmitter, released from presynaptic terminals, produces an electrophysiological response in the postsynaptic cell is conceived as follows. The released neurotransmitter activates a neurotransmitter-sensitive adenylate cyclase present in the membrane of the postsynaptic cell, leading to the production of cyclic AMP in the immediate vicinity of the postsynaptic membrane. The newly formed cyclic AMP activates a cyclic AMP-dependent protein kinase present in the postsynaptic membrane. This activated protein kinase catalyses the phosphorylation of a substrate protein also present in the postsynaptic membrane, converting it from the non-phosphorylated to the phosphorylated state. A key element of this model is that this substrate protein controls the permeability of the postsynaptic membrane. Phosphorylation of the substrate protein leads, through a change either in ion conductance or in the rate of an electrogenic pump, to a change in membrane potential, the "postsynaptic potential". Since the postsynaptic potential is transient in nature, enzymatic machinery must exist which terminates this sequence of events. This enzymatic machinery includes a phosphodiesterase that hydrolyses the cyclic AMP to 5'-AMP and a phosphoprotein phosphatase that converts the substrate protein back to the non-phosphorylated form, leading to the termination of the postsynaptic potential.

as the rate at which these microtubules transport material along their length, or the rate of assembly or disassembly of the microtubules.

The third possible role, which is the main theme of this presentation, is in mediating the postsynaptic effects of neurotransmitters at certain types of synapse. An hypothetical sequence of events by which this may occur is presented in Fig. 3. Although the scheme is far from proven, a variety of evidence is compatible with the idea that the effects of cyclic AMP on the membrane properties of postsynaptic cells may be mediated through regulation of the state of phosphorylation of specific membrane proteins. I shall now summarise some of the evidence bearing on this issue.

The five proteins indicated in the postsynaptic neurone in Fig. 3, namely adenylate cyclase⁷⁶, phosphodiesterase⁷⁶⁻⁷⁸, protein kinase⁷⁹, protein kinase substrate⁸⁰, and phosphoprotein phosphatase⁸¹, are present in very high concentrations in those subcellular fractions of brain tissue that are richest in synaptic membranes. Cyclic AMP markedly stimulates the phosphorylation, by endogenous protein kinase, of two substrate proteins, designated protein I and protein II, present in these synaptic membrane fractions (Fig. 4)⁸². The phosphorylation of both proteins reaches a maximum within 5 s of incubation, the shortest reaction time that has been studied. Since the molecular events underlying synaptic transmission must be rapid, the rapid phosphorylation of these proteins is almost a prerequisite to considering seriously the possibility that one or another of them might be involved in the generation of postsynaptic potentials. Studies of these synaptic membrane proteins have yielded some provocative results.

Studies of the subcellular distribution of protein I indicate large amounts are present in synaptic membranes⁸². In contrast, protein I has not been found in any subcellular fraction of any non-nervous tissue examined⁸². Two other studies of protein I, now in progress in our laboratory, can be mentioned briefly. First, Suzanne Lohmann has obtained evidence that the amount of protein I is very low in neonatal rat cerebrum, and that it increases sharply 2-3 weeks postnatally, when there is also a marked increase in histochemically demonstrable synaptic structures⁸³. Secondly, Tetsufumi Ueda has succeeded in solubilising protein I from a synaptic membrane fraction of bovine brain, and has purified it approximately 1,000-fold, calculated with reference to the solubilised extract. Starting with 1.3 kg of brain, he has obtained about 900 µg of apparently pure protein I. It should be possible to collect enough protein I to carry out a study of its structure and of its physicochemical properties, and to determine whether phosphorylation alters these properties. Such studies could provide clues to the chemical basis for the physiological changes observed in some postsynaptic membranes during synaptic transmission. Purified protein I, in its non-phosphorylated and phosphorylated forms, could also be added to model membranes to see if either form affects their permeability.

Moreover, antibodies to purified protein I are now being prepared with several aims in mind, including developing a sensitive radioimmunoassay for protein I, examining the possible effect of the antibodies on neuronal function, and determining the cytochemical distribution of protein I. It will be of interest to see whether such an immunocytochemical approach verifies the localisation of protein I in the postsynaptic membrane.

In contrast to the apparently unique localisation of protein I in neuronal tissue, protein II, or at least a protein with a number of properties similar to those of protein II, is present in membranes and cytosol of a variety of tissues. Certain properties of the protein II system suggest that it could be involved in molecular events underlying a variety of rapid physiological processes. For instance, the rate of phosphorylation and the rate of dephosphorylation of protein II are both stimulated by cyclic AMP⁸⁴. Such a mechanism, while thermodynamically unappealing since the overall effect is to hydrolyse ATP, has the asset of providing a mechanism for a very short-lived pulse

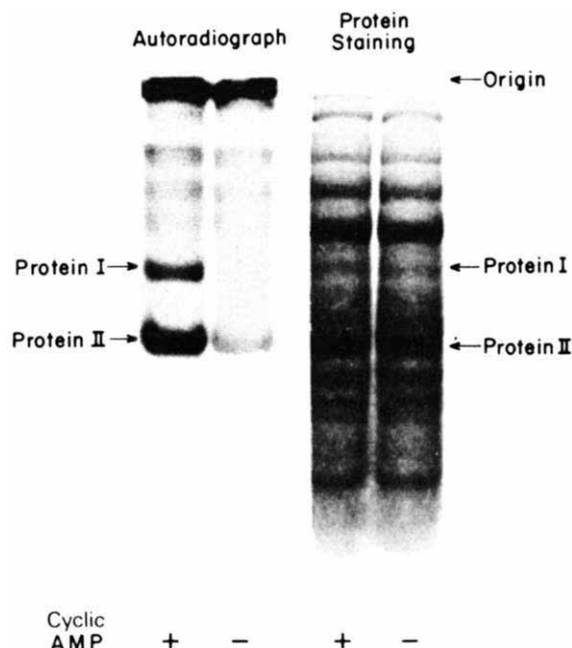


Fig. 4 Effect of cyclic AMP on the phosphorylation of endogenous membrane proteins in a synaptic membrane preparation from rat cerebrum. The synaptic membrane preparation was incubated with γ -³²P-ATP, in the absence or presence of cyclic AMP, for 15 s. The reaction was stopped by the addition of sodium dodecyl sulphate. The solubilised protein was then subjected to slab gel electrophoresis, protein staining, and autoradiography, to determine those protein bands into which radioactive phosphate had been incorporated. Autoradiograph is shown on the left side, and a photograph of the protein staining pattern of the dried gel is shown on the right side. The + and - signs at the bottom of the gel indicate the presence and absence, respectively, of 5×10^{-6} M cyclic AMP in the incubation mixture. Several dozen protein bands are visible in the protein staining pattern. A few of these bands incorporated radioactive phosphate, as indicated by the autoradiograph. Phosphorylation of two protein bands was markedly affected by cyclic AMP. The arrows identify the positions on the gel of these two protein bands, designated protein I and protein II, the apparent molecular weights of which are 86,000 and 48,000, respectively. (Taken from Ueda *et al.*⁸².)

of phosphorylated protein. Recently, the protein II kinase-protein II-protein II phosphatase system has been solubilised from synaptic membranes⁸⁵. A kinetic study indicates that the three proteins are present in the solubilised system, and presumably are present in the intact synaptic membranes, in the form of a complex. The existence of the three protein components of a protein phosphorylation-dephosphorylation system in the form of a complex in the synaptic membrane would overcome one theoretical problem associated with the idea that phosphorylation of membrane proteins may mediate the actions of some neurotransmitters. Thus, if the three proteins were separate from one another, and it were necessary to rely on diffusion for the substrate protein to interact with its kinase and phosphatase, the reaction rates might be too slow to underlie events as rapid as those associated with the generation of a postsynaptic potential. By being in the form of a complex, the system is not limited by rates of diffusion and, therefore, the phosphorylation and dephosphorylation steps could be extremely rapid. Studies are in progress to determine whether the protein I system also exists as a complex in synaptic membranes.

It would be helpful, in evaluating the scheme shown in Fig. 3, to determine whether a correlation exists, in an appropriate intact neuronal preparation, between the time course of the phosphorylation and dephosphorylation of some membrane protein and the time course of the rise and decay of the postsynaptic potential. But the electrophysiological and the

biochemical responses in neuronal tissue are so rapid that it is not feasible to carry out such a study at present. Cyclic nucleotides also are involved in regulating membrane function in many non-neuronal tissues. Fortunately, certain of these non-neuronal preparations have been found useful for testing the hypothesis that the demonstrated ability of cyclic AMP to regulate the permeability of cell membranes is mediated through control of the state of phosphorylation of specific membrane proteins. The results have proved of interest both for understanding regulation of membrane function in non-neuronal tissues, as well as for their possible relevance to an understanding of brain function. Some of the results obtained with such preparations will be summarised next.

Cyclic AMP, protein phosphorylation, and membrane permeability in non-neuronal systems

β -Adrenergic agonists, including isoproterenol, adrenaline, and noradrenaline, cause an increase in sodium and potassium fluxes across the plasma membranes of avian erythrocytes⁸⁶⁻⁸⁸. There is evidence^{87,88} that cyclic AMP mediates this physiological response: β -adrenergic agonists increase the concentration of cyclic AMP in these cells and cyclic AMP mimics the effect of the β -adrenergic agents in increasing cation fluxes. Moreover, the response is slow enough to permit measurements of protein phosphorylation at various times in the course of the response. Experiments were therefore carried out with intact turkey erythrocytes preincubated with radioactive inorganic phosphate, to label the intracellular ATP pool, and then incubated in the presence of various test substances⁸⁹. Exposure of intact turkey erythrocytes to β -adrenergic agonists for a period of a few minutes markedly affected the degree of phosphorylation of only one protein. This protein had an apparent molecular weight of 240,000 and was located in the plasma membrane. In a variety of experimental conditions, there was a good correlation between the phosphorylation of this membrane protein and the cation flux across the membrane.

The toad bladder is another preparation which has been used to explore the possibility that cyclic AMP may regulate membrane permeability by controlling the state of phosphorylation of a specific protein. Antidiuretic hormone (ADH) increases the sodium permeability of the apical membrane of toad bladder epithelium by a mechanism which seems to involve cyclic AMP. Thus, ADH causes an increase in the concentration of cyclic AMP in this epithelium and cyclic AMP mimics the effect of ADH in increasing sodium transport⁹⁰. Using intact epithelial cells, a correlation has been found between the degree of phosphorylation of a specific protein, called protein D, and the rate of sodium transport, in a variety of experimental conditions in which the cyclic AMP levels were manipulated^{91,92}. In addition, aldosterone, which causes a delayed increase in sodium transport across toad bladder epithelium, but apparently not by altering the concentration of cyclic AMP, also affects the degree of phosphorylation of protein D⁹³. The effects of aldosterone, both on protein D phosphorylation and on sodium permeability, require *de novo* RNA and protein synthesis, whereas the corresponding effects of ADH, mediated through cyclic AMP, do not. Thus, both ADH and aldosterone regulate the sodium permeability of this tissue and also effect the phosphorylation of the same specific protein, although by different mechanisms.

Studies of a membrane fraction derived from the sarcoplasmic reticulum of myocardial cells have provided evidence that phosphorylation of a specific protein may be involved in regulation by cyclic AMP of Ca^{2+} transport across intracellular membranes in the heart⁹⁴⁻⁹⁶. The sarcoplasmic reticulum is believed to represent an uptake system for removing Ca^{2+} from the vicinity of the contractile components of muscle fibres. Cyclic AMP stimulates the active transport of Ca^{2+} into isolated vesicles derived from the sarcoplasmic reticulum. This effect of cyclic AMP may be a component of the mechanism by which β -adrenergic agonists shorten the duration of cardiac

contraction. On addition of cyclic AMP to these isolated vesicles, a correlation is observed between increased rate of Ca^{2+} transport, stimulation of a Ca^{2+} - Mg^{2+} -dependent ATPase, and phosphorylation of a 22,000-dalton membrane protein⁹⁴⁻⁹⁶. This phosphorylated protein may act as a modulator of the Ca^{2+} - Mg^{2+} ATPase^{95,96}.

The results obtained with several biological systems studied to date are compatible with the proposal that regulation of the phosphorylation of specific membrane proteins is involved in the mechanism by which cyclic AMP alters ion transport across membranes. It must be emphasised however, that what has been demonstrated is correlation but not causality. The development of highly specific activators and inhibitors of membrane-bound protein kinases and protein phosphatases would be of substantial help in evaluating critically the nature of the relationship between protein phosphorylation and membrane permeability.

Cyclic GMP-dependent protein phosphorylation in smooth muscle

Recent studies with mammalian smooth muscle suggest that the effects of cyclic GMP on postsynaptic membranes, like those of cyclic AMP, may be mediated through regulation of the phosphorylation of specific membrane proteins. ACh, acting on muscarinic receptors, causes a rapid and large increase in cyclic GMP in smooth muscle^{97,98}. A protein kinase specifically activated by low concentrations of cyclic GMP, rather than cyclic AMP, as well as endogenous substrate proteins for this cyclic GMP-dependent protein kinase, have been found in a membrane fraction from mammalian smooth muscle⁹⁹. As shown in Fig. 5, a half-maximal increase in the degree of phosphorylation of two substrate proteins, designated protein GI and protein GII, catalysed by an endogenous protein kinase, occurred at about 4×10^{-8} M cyclic GMP, in a membrane fraction from the ductus deferens. A 10- to 20-fold higher concentration of cyclic AMP was required to stimulate phosphorylation of these membrane proteins to the same extent. Cyclic GMP-dependent phosphorylation of proteins GI and GII has been found in a membrane fraction from all types of smooth muscle examined⁹⁹. In contrast, proteins GI and GII have not been found in the cell sap of smooth muscle nor have they been found in any particulate or soluble fraction of a number of other tissues examined. Thus, it would seem that proteins GI and GII are specific to certain membranes from smooth muscle. The fact that ACh increases the level of cyclic GMP in smooth muscle, and that low concentrations of cyclic GMP stimulate the phosphorylation of two proteins which seem to be specific to smooth muscle membranes, suggests that cyclic GMP, as well as proteins GI and GII, may be involved in mediating or modulating some of the physiological effects of ACh on smooth muscle.

Although cyclic GMP-dependent protein kinases were discovered several years ago¹⁰⁰⁻¹⁰², and have been demonstrated in a variety of tissues throughout the animal kingdom¹⁰⁰⁻¹⁰⁶ including mammalian brain^{101,103-105}, smooth muscle is the only tissue in which any endogenous substrate proteins for this class of enzymes have yet been found. Demonstration of cyclic GMP-dependent phosphorylation of endogenous substrate proteins in neuronal tissue, by endogenous protein kinases, would be of considerable help in understanding the role and mechanism of action of cyclic GMP in the nervous system.

Multiple roles for cyclic nucleotides and protein phosphorylation in neuronal function

The data summarised in this article suggest that the postsynaptic actions of a number of neurotransmitters may be mediated through cyclic AMP or cyclic GMP. Moreover, it is proposed that the immediate targets of cyclic AMP and cyclic GMP are protein kinases whose activation results in the phosphorylation of specific proteins in the membranes of postsynaptic cells, and that the alteration of the degree of

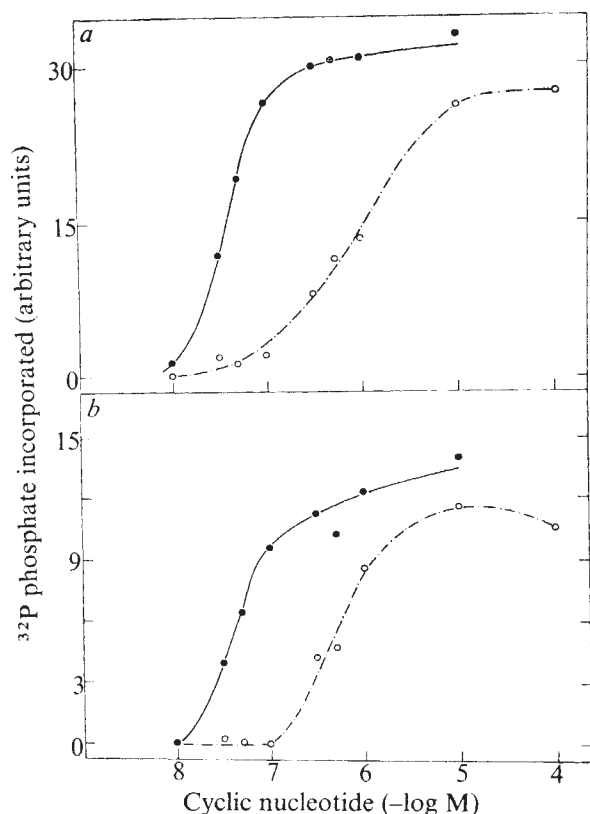


Fig. 5 Effect of various concentrations of cyclic GMP and cyclic AMP on the endogenous phosphorylation of proteins in a membrane preparation from guinea pig ductus deferens. The membrane preparation was incubated for 15 s with γ - ^{32}P -ATP in the absence or presence of cyclic GMP ($\bullet$; $K_a = 4 \times 10^{-8}$ M (a) or 5×10^{-8} M (b)) or cyclic AMP ($\circ$; $K_a = 8 \times 10^{-7}$ M (a) or 5×10^{-7} M (b)). The reaction was stopped by the addition of sodium dodecyl sulphate, and the solubilised protein was subjected to slab gel electrophoresis and autoradiography. Cyclic GMP markedly affected the phosphorylation of two protein bands, designated protein G1 (a) and protein GII (b), with apparent molecular weights of 130,000 and 100,000, respectively. Incorporation of radioactive phosphate into these proteins was measured quantitatively by densitometric analysis of the autoradiograms. Results of those measurements are shown here. The data represent the increase in phosphorylation above that observed in the absence of added cyclic nucleotide, which amounted to 9.8 and 1.4 arbitrary units for protein G1 and protein GII, respectively. (Taken from Casnellie and Greengard⁹⁹.)

phosphorylation of these membrane proteins produces the observed physiological effects of the neurotransmitters. It should, however, be emphasised that cyclic nucleotides and phosphorylated proteins may have many other roles in the nervous system. Two such roles include regulation of neurotransmitter synthesis and microtubular function, as discussed briefly above. The increase in cyclic AMP during nervous activity may also serve to mobilise carbohydrate and lipid reserves and thus to meet the increased energy demands associated with elevated functional activity. Cyclic AMP formed in response to neurotransmitters probably induces the synthesis *de novo* of a number of proteins with specific functional roles, including tyrosine hydroxylase¹⁰⁷⁻¹⁰⁹ and *N*-acetyl transferase^{110,111}. Finally, a 5-HT-mediated increase in cyclic AMP may be responsible for the increased neurotransmitter release that accompanies behavioural sensitisation in certain invertebrate nerve networks^{112,113}.

The biochemical mechanisms by which phenomena as varied as energy mobilisation, axonal transport, synaptic transmission, synthesis and activation of enzymes controlling neurotransmitter formation, and short term and long term information storage and retrieval are regulated in an orderly and coordinated fashion represent a challenging topic for future research in

neurobiology. It seems likely that answers to many of the questions in this area will be found through understanding the regulatory and integrative role of cyclic nucleotides and phosphorylated proteins in the nervous system.

- 1 Robison, G. A., Butcher, F. R. W., and Sutherland, E. W., *Cyclic AMP* (Academic, New York, 1971).
- 2 Beam, K. G., and Greengard, P., in *Cold Spring Harb. Symp. quant. Biol.* (in the press).
- 3 Eccles, R., and Libet, B., *J. Physiol., Lond.*, **157**, 484 (1961).
- 4 Volle, R. L., in *Muscarinic and Nicotinic Stimulant Actions at Autonomic Ganglia*, (Pergamon, New York, 1966).
- 5 Libet, B., *Fedn Proc.*, **29**, 1945 (1970).
- 6 Greengard, P., and McAfee, D. A., *Br. biochem. Soc. Symp.*, **36**, 87 (1972).
- 7 Greengard, P., and Keibabian, J. W., *Fedn Proc.*, **33**, 1059 (1974).
- 8 Kalix, P., McAfee, D. A., Schorderet, M., and Greengard, P., *J. pharmac. exp. Ther.*, **188**, 676 (1974).
- 9 McAfee, D. A., Schorderet, M., and Greengard, P., *Science*, **171**, 1156 (1971).
- 10 Keibabian, J. W., and Greengard, P., *Science*, **174**, 1346 (1971).
- 11 Keibabian, J. W., Bloom, F. E., Steiner, A. L., and Greengard, P., *Science*, **190**, 157 (1975).
- 12 McAfee, D. A., and Greengard, P., *Science*, **178**, 310 (1972).
- 13 Keibabian, J. W., Steiner, A. L., and Greengard, P., *J. pharmac. exp. Ther.*, **193**, 474 (1975).
- 14 Eccles, R. M., *J. Physiol., Lond.*, **117**, 196 (1952).
- 15 Weight, F. F., Petzold, G., and Greengard, P., *Science*, **186**, 942 (1974).
- 16 Siggins, G. R., Hoffer, B. J., and Bloom, F. E., *Brain Res.*, **25**, 535 (1971).
- 17 Siggins, G. R., Oliver, A. P., Hoffer, B. J., and Bloom, F. E., *Science*, **171**, 192 (1971).
- 18 Hoffer, B. J., Siggins, G. R., Oliver, A. P., and Bloom, F. E., *J. pharmac. exp. Ther.*, **184**, 553 (1973).
- 19 Bloom, F. E., Siggins, G. R., Hoffer, B. J., Segal, M., and Oliver, A. P., in *Advances in Cyclic Nucleotide Research*, 5 (edit. by Drummond, G. I., Greengard, P., and Robison, G. A.), 603 (Raven, New York, 1975).
- 20 Carlsson, A., and Lindquist, M., *Acta pharmac. tox.*, **20**, 140 (1963).
- 21 Hornykiewicz, O., *Pharmac. Rev.*, **18**, 925 (1966).
- 22 Andén, N.-E., Rubenson, A., Fuxe, K., and Hökfelt, T., *J. Pharm. Pharmac.*, **19**, 627 (1967).
- 23 Nybäck, H., and Sedvall, G., *J. pharmac. exp. Ther.*, **162**, 294 (1968).
- 24 Ungerstedt, U., Butcher, L. L., Butcher, S. G., Andén, N.-E., and Fuxe, K., *Brain Res.*, **14**, 461 (1969).
- 25 Nybäck, H., Schubert, J., and Sedvall, G., *J. Pharm. Pharmac.*, **22**, 622 (1970).
- 26 Corrodi, H., Fuxe, K., and Ungerstedt, U., *J. Pharm. Pharmac.*, **23**, 989 (1971).
- 27 Hornykiewicz, O., *Contemp. Neurol.*, **8**, 34 (1971).
- 28 Andén, N.-E., *J. Pharm. Pharmac.*, **24**, 905 (1972).
- 29 Nybäck, H., and Sedvall, G., *Psychopharmac.*, **26**, 155 (1972).
- 30 Bunney, B. S., Walters, J. R., Roth, R. H., and Aghajanian, G. K., *J. pharmac. exp. Ther.*, **185**, 560 (1973).
- 31 Randrup, A., and Munkvad, I., *Orthomolec. Psychiat.*, **1**, 2 (1972).
- 32 Matthysse, S., *Fedn Proc.*, **32**, 200 (1973).
- 33 Stevens, J., *Archs gen. Psychiat.*, **29**, 177 (1973).
- 34 Snyder, S. H., Banerjee, S. P., Yamamura, H. I., and Greengard, D., *Science*, **184**, 1243 (1974).
- 35 Brown, J. H., and Makman, M. H., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 539 (1972).
- 36 Keibabian, J. W., Petzold, G. L., and Greengard, P., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2145 (1972).
- 37 Clement-Cormier, Y. C., Keibabian, J. W., Petzold, G. L., and Greengard, P., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1113 (1974).
- 38 Miller, R. J., Horn, A. S., and Iversen, L. L., *Molec. Pharmac.*, **10**, 759 (1974).
- 39 Karobath, M., and Leitch, H., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2915 (1974).
- 40 Miller, R., Horn, A., Iversen, L., and Pinder, R., *Nature*, **250**, 238 (1974).
- 41 Sheppard, H., and Burghardt, C. R., *Molec. Pharmac.*, **10**, 721 (1974).
- 42 Makman, M. H., Brown, J. H., and Mishra, R. K., in *Advances in Cyclic Nucleotide Research*, 5 (edit. by Drummond, G. I., Greengard, P., and Robison, G. A.), 661 (Raven, New York, 1975).
- 43 Mishra, R. K., Demirjian, C., Katzman, R., and Makman, M. H., *Brain Res.*, **96**, 395 (1975).
- 44 Iversen, L. L., *Science*, **188**, 1084 (1975).
- 45 von Hungen, K., Roberts, S., and Hill, D. F., *Brain Res.*, **94**, 57 (1975).
- 46 Clement-Cormier, Y. C., Parrish, R. G., Petzold, G. L., Keibabian, J. W., and Greengard, P., *J. Neurochem.*, **25**, 143 (1975).
- 47 Rall, T. W., *Ann. N.Y. Acad. Sci.*, **185**, 520 (1971).
- 48 Drummond, G. I., and Ma, Y., in *Progress in Neurobiology*, 2 (edit. by Kerkut, G. A., and Phillis, J. W.), 119 (Pergamon, New York, 1973).
- 49 Nathanson, J. A., and Greengard, P., *Science*, **180**, 308 (1973).
- 50 Greengard, P., Nathanson, J. A., and Keibabian, J. W., in *Frontiers in Catecholamine Research* (edit. by Usdin, E., and Snyder, S.), 377 (Pergamon, London, 1973).
- 51 von Hungen, K., and Roberts, S., in *Reviews of Neuroscience*, 1 (edit. by Ehrenpreis, S., and Kopin, I. J.), 231 (Raven, New York, 1974).
- 52 Bloom, F. E., in *Reviews of Physiology, Biochemistry, and Experimental Pharmacology* (Springer, Berlin, in the press).
- 53 Daly, J. W., in *Handbook of Psychopharmacology*, 5 (edit. by Iversen, L. L., Iversen, S. H., and Snyder, S. H.), 47 (Plenum, New York, 1975).
- 54 Nathanson, J. A., and Greengard, P., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 797 (1974).
- 55 von Hungen, K., Roberts, S., and Hill, D. F., *Nature*, **252**, 588 (1974).
- 56 Collier, H. O. J., and Roy, A. C., *Nature*, **248**, 24 (1974).
- 57 Sharma, S. K., Nirenberg, M., and Klee, W. A., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 590 (1975).
- 58 Traber, J., Reiser, G., Fischer, K., and Hamprecht, B., *FEBS Lett.*, **52**, 327 (1975).
- 59 Ferrendelli, J. A., Steiner, A. L., McDougal, D. R., and Kipnis, D. M., *Biochem. biophys. Res. Commun.*, **41**, 1061 (1970).
- 60 Kuo, J. F., Lee, T.-P., Reyes, P. L., Walton, K. G., Donnelly, T. E., Jr., and Greengard, P., *J. biol. Chem.*, **247**, 16 (1972).
- 61 Stone, T. W., Taylor, D. A., and Bloom, F. E., *Science*, **187**, 845 (1975).
- 62 Kuo, J. F., and Greengard, P., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 1349 (1969).
- 63 Walsh, D. A., Perkins, J. P., and Krebs, E. G., *J. biol. Chem.*, **243**, 3763 (1968).
- 64 Langan, T. A., *Science*, **162**, 579 (1968).
- 65 Miyamoto, E., Kuo, J. F., and Greengard, P., *J. biol. Chem.*, **244**, 6395 (1969).
- 66 Goldstein, M., Anagnoste, B., and Shiron, C., *J. Pharm. Pharmac.*, **25**, 348 (1973).
- 67 Harris, J. E., Baldessarini, R. J., and Wheeler, S., *Fedn Proc.*, **33**, 521 (1974).
- 68 Roberge, C., Ebstein, B., and Goldstein, M., *Fedn Proc.*, **33**, 521 (1974).
- 69 Harris, J. E., Baldessarini, R. J., Morgenroth, V. H., III, and Roth, R. H., *Nature*, **252**, 156 (1974).
- 70 Morgenroth, V. H., III, Hegstrand, L. R., Roth, R. H., and Greengard, P., *J. biol. Chem.*, **250**, 1946 (1975).
- 71 Lovenberg, W., Bruckwick, E. A., and Hanbauer, I., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2955 (1975).
- 72 Bronaugh, R. L., Kashimoto, T., and Goldstein, M., *Neurosci. Abstr.*, **547** (1975).
- 73 Lloyd, T., and Kaufman, S., *Biochem. biophys. Res. Commun.*, **66**, 907 (1975).

- 74 Goodman, D. B. P., Rasmussen, H., DiBella, F., and Guthrow, C. E., Jr, *Proc. natn. Acad. Sci. U.S.A.*, **67**, 652 (1970).
- 75 Sloboda, R. D., Rudolph, S. A., Rosenbaum, J. L., and Greengard, P., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 177 (1975).
- 76 DeRobertis, E., DeLores Arnaiz, G. R., Alberici, M., Butcher, R. W., and Sutherland, E. W., *J. biol. Chem.*, **242**, 3487 (1967).
- 77 Cheung, W. Y., and Salganicoff, L., *Nature*, **214**, 90 (1967).
- 78 Florendo, N. T., Barnett, R. J., and Greengard, P., *Science*, **173**, 745 (1971).
- 79 Maeno, H., Johnson, E. M., and Greengard, P., *J. biol. Chem.*, **246**, 134 (1971).
- 80 Johnson, E. M., Maeno, H., and Greengard, P., *J. biol. Chem.*, **246**, 7731 (1971).
- 81 Maeno, H., and Greengard, P., *J. biol. Chem.*, **247**, 3269 (1972).
- 82 Ueda, T., Maeno, H., and Greengard, P., *J. biol. Chem.*, **248**, 8295 (1973).
- 83 Aghajanian, G. K., and Bloom, F. E., *Brain Res.*, **6**, 716 (1967).
- 84 Maeno, H., Ueda, T., and Greengard, P., *J. cyclic Nucl. Res.*, **1**, 37 (1975).
- 85 Ueda, T., Rudolph, S. A., and Greengard, P., *Archs Biochem. Biophys.*, **170**, 492 (1975).
- 86 Kregenow, F. M., *J. gen. Physiol.*, **61**, 509 (1973).
- 87 Riddick, D. H., Kregenow, F. M., and Orloff, J., *J. gen. Physiol.*, **57**, 752 (1971).
- 88 Gardner, J. D., Klaevman, H. L., Bilezikian, J. P., and Aurbach, G. D., *J. biol. Chem.*, **248**, 5590 (1973).
- 89 Rudolph, S. A., and Greengard, P., *J. biol. Chem.*, **249**, 5684 (1974).
- 90 Orloff, J., and Handler, J., *Am. J. Med.*, **42**, 757 (1967).
- 91 DeLorenzo, R. J., Walton, K. G., Curran, P. F., and Greengard, P., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 880 (1973).
- 92 Walton, K. G., DeLorenzo, R. J., Curran, P. F., and Greengard, P., *J. gen. Physiol.*, **65**, 153 (1975).
- 93 Liu, A. Y.-C., and Greengard, P., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3869 (1974).
- 94 Tada, M., Kirchberger, M. A., Repke, D. I., and Katz, A. M., *J. biol. Chem.*, **249**, 6174 (1974).
- 95 LaRaia, P. J., and Morkin, E., *Circ. Res.*, **35**, 298 (1974).
- 96 Tada, M., Kirchberger, M. A., and Katz, A. M., *J. biol. Chem.*, **250**, 2640 (1975).
- 97 Lee, T.-P., Kuo, J. F., and Greengard, P., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3287 (1972).
- 98 Schultz, G., Hardman, J. G., Schultz, K., Davis, J. W., and Sutherland, E. W., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1721 (1973).
- 99 Casnellie, J. E., and Greengard, P., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1891 (1974).
- 100 Kuo, J. F., and Greengard, P., *J. biol. Chem.*, **245**, 2493 (1970).
- 101 Greengard, P., and Kuo, J. F., in *Role of Cyclic AMP in Cell Function* (edit. by Greengard, P., and Costa, E.), *Advances in Biochemical Psychopharmacology*, **3**, 287 (Raven, New York, 1970).
- 102 Kuo, J. F., Wyatt, G. R., and Greengard, P., *J. biol. Chem.*, **246**, 7159 (1971).
- 103 Hofmann, F., and Söld, G., *Biochem. Biophys. Res. Commun.*, **49**, 1100 (1972).
- 104 Kuo, J. F., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2256 (1975).
- 105 Takai, Y., Nishiyama, K., Yamamura, H., and Nishizuka, Y., *J. biol. Chem.*, **250**, 4690 (1975).
- 106 Nakazawa, K., and Sano, M., *J. biol. Chem.*, **250**, 7415 (1975).
- 107 Waymire, J. C., Weiner, N., and Prasad, K. N., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2241 (1972).
- 108 Richelson, F., *Nature new Biol.*, **242**, 175 (1973).
- 109 Costa, E., Guidotti, A., and Hanbauer, I., *Life Sci.*, **14**, 1169 (1974).
- 110 Klein, D. C., and Berg, G. R., in *Role of Cyclic AMP in Cell Function* (edit. by Greengard, P., Costa, E.), *Advances in Biochemical Psychopharmacology*, **3**, 241 (Raven, New York, 1970).
- 111 Romero, J. A., Zatz, M., and Axelrod, J., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2107 (1975).
- 112 Kandel, E. R., Brunelli, M., Byrne, J., and Castellucci, V., in *Cold Spring Harb. Symp. quant. Biol.* (in the press).
- 113 Brunelli, M., Castellucci, V., and Kandel, E. R., *Neurosci. Abstr.*, **568** (1975)

articles

Laboratory investigation of tilt and seismicity anomalies in rock before failure

B. T. Brady

US Department of the Interior, Bureau of Mines, Denver Mining Research Center, Denver, Colorado 80225

Laboratory measurements of tilt and seismicity anomalies whose time durations are a few milliseconds are shown to precede failure in dry rock specimens. The observed tilt and seismicity behaviour is qualitatively similar to that reported to precede earthquakes. Test results suggest that this precursor time for laboratory specimens of this length may be independent of rock type, in agreement with theoretical predictions and other experimental data on rock failures including earthquakes.

A SCALE invariant theory of rock failure has been proposed that predicts precursor effects such as anomalous V_p/V_s and V_p/V_s behaviour and tilt before failure in rock². In addition, because the theory does not vary with scale, these effects are predicted to occur on the laboratory scale over a time interval of a few milliseconds, and that this time interval will be independent of rock type^{3,4}. Experimental evidence is presented below that indicates that tilt and seismicity precursors, whose characteristics are qualitatively similar to those observed before earthquakes, are observed a few milliseconds before failure of rock on the laboratory scale. Preliminary experimental results suggest this time interval may be independent of rock type.

Experimental procedure

Cylindrical samples of 5.39 cm diameter of a variety of rock types including Barre granite and Berea sandstone (reported here), oil shale, schist and marble were drilled, cut and ground

to a final length of 13.5 cm. All specimens were deformed to failure under a confining pressure of 1.5×10^7 Pa (10^5 Pa = 1 bar) in a servo-controlled materials testing system. Tests were performed under deformation control with a displacement rate of 6.5×10^{-4} cm s⁻¹.

The load cell used in the testing programme was unique as both the x - and y -moments (M_x and M_y , respectively), in addition to the total load, F (z axis is load direction), could be measured and recorded on separate channels as a function of time. Bending moments, similar to tilts measured in regions prone to earthquakes, are measured about two perpendicular axes intersecting at the centre of the transducer and normal to the load axis. Moment and load changes were recorded on a visicorder and/or a digital memory scope (details of this equipment are available elsewhere⁵).

Acoustic emission, or equivalently, seismicity from the test samples was measured with a system (Dunegan/Endevco 3000) that included a log converter (model 60) and a reset clock (model 402). The reset clock was modified to provide precision tuning pulses with a minimum pulse interval of 200 μ s. The log converter produces a logarithmic scaling of both total emission and emission rate. A low mass ($\lesssim 1$ g) piezoelectric transducer with a ring-down time of ~ 20 μ s was used to detect seismic emission from the test samples.

Experimental data

Figure 1 illustrates the variation with time of bending moment (M_x and M_y) for Barre granite (BG-T28) and Berea sandstone (BSS-5). Visicorder output of M_x and M_y as a function of time is shown in Fig. 1b. Photographs of both the time variation of

M_x and M_y for the first fracture in Berea sandstone (recorded on the digital oscilloscope) and the fractured test samples are shown in Fig. 1a and c, respectively. Note that the failure of the Barre granite specimen was a multiple (2) fracture event with the second fracture developing ~ 85 ms after the initial break. The failure of the Berea sandstone specimen, on the other hand, was a single fracture event. This observation should not be construed as suggesting that the existence of multiple or single fractures in failed rock is a function of rock type. Based on our tests, the occurrence of multiple compared with single fractures in failed rock depends only on such parameters as confining

pressure, uniformity of loading and the existence of local inhomogeneities within the specimen. Moment and total load (F) variation with time for Barre granite specimens T14, T29 and T30 are displayed in the first column of Fig. 2a-c. Specimens T14 (Fig. 2a) and T30 (Fig. 2c) are single fracture events. Specimen T29 was a multiple (2) event. Moment and load changes for the first fracture in T29 are shown in Fig. 2b2 and b3.

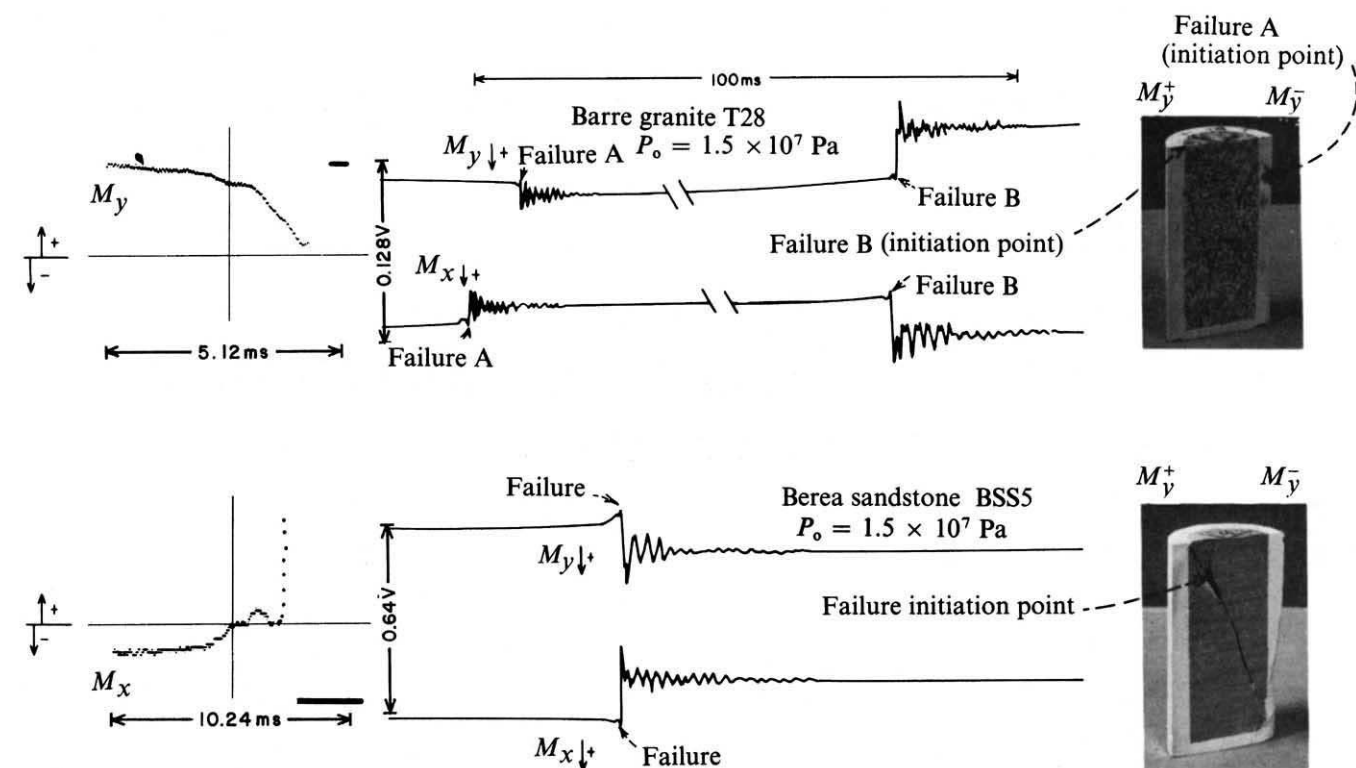


Fig. 1 Variation of M_y and M_x with time for Barre granite (BG-T28) and Berea sandstone (BSS-5). *a*, Photographs of digital oscilloscope output for moment variation. The conversion factor for the vertical scale (in volts) is $3.46 \times 10^3 \text{ N m V}^{-1}$. *b*, Visicorder output of M_y and M_x against time; *c*, photographs of fractured test samples showing major fault zones.

pressure, uniformity of loading and the existence of local inhomogeneities within the specimen. Moment and total load (F) variation with time for Barre granite specimens T14, T29 and T30 are displayed in the first column of Fig. 2a-c. Specimens T14 (Fig. 2a) and T30 (Fig. 2c) are single fracture events. Specimen T29 was a multiple (2) event. Moment and load changes for the first fracture in T29 are shown in Fig. 2b2 and b3.

The most prominent feature in both Figs 1 and 2 is tilting in the direction of eventual fault growth a few milliseconds before failure. This is followed by a reversal in tilt direction that culminates in specimen failure. The inferred initiation locations for failure (based on these moment changes) were substantiated by microscopic examination that showed increased crack concentrations in these locations (Fig. 1c). Note also that the total load in samples T29 and T30 decreases during this interval: the magnitudes of both the load decrease and moment changes within these specimens during this interval are $\sim 2,500 \text{ N}$ and 100 Nm , respectively. Also, the times of the tilt and load change anomalies are nearly identical. The tilt anomaly is, however, observed always to precede the load change, in this case, load

decrease, by a time that is usually $< 1 \text{ ms}$. Since the load changes within the specimen occur over such a small time, and because the primary hydraulic loading system of the machine cannot respond to deformation changes in $\lesssim 100 \text{ ms}$, load change within the specimen is transferred to the columns of the test machine. Thus, the sample continues to shorten during this time. It is not difficult to show theoretically that in these conditions the post-failure stiffness of the sample decreases during the anomaly time interval. Catastrophic failure occurs only when the post-failure stiffness of the sample equals the loading system stiffness³.

Discussion

A scale invariant theory of failure governing the initiation and growth of faulting in rock has been proposed². The scale invariant properties of failing rock provide an explanation of

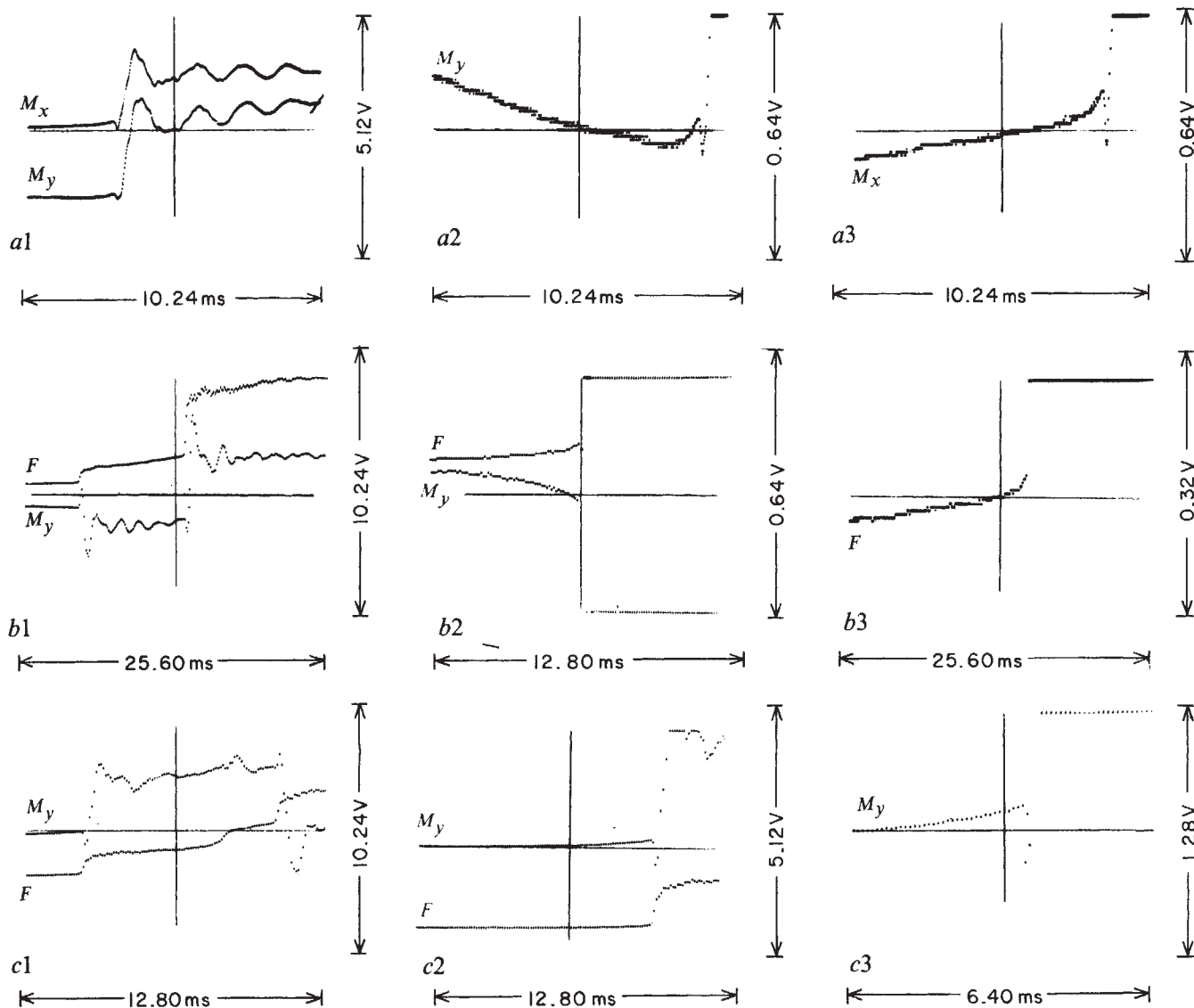
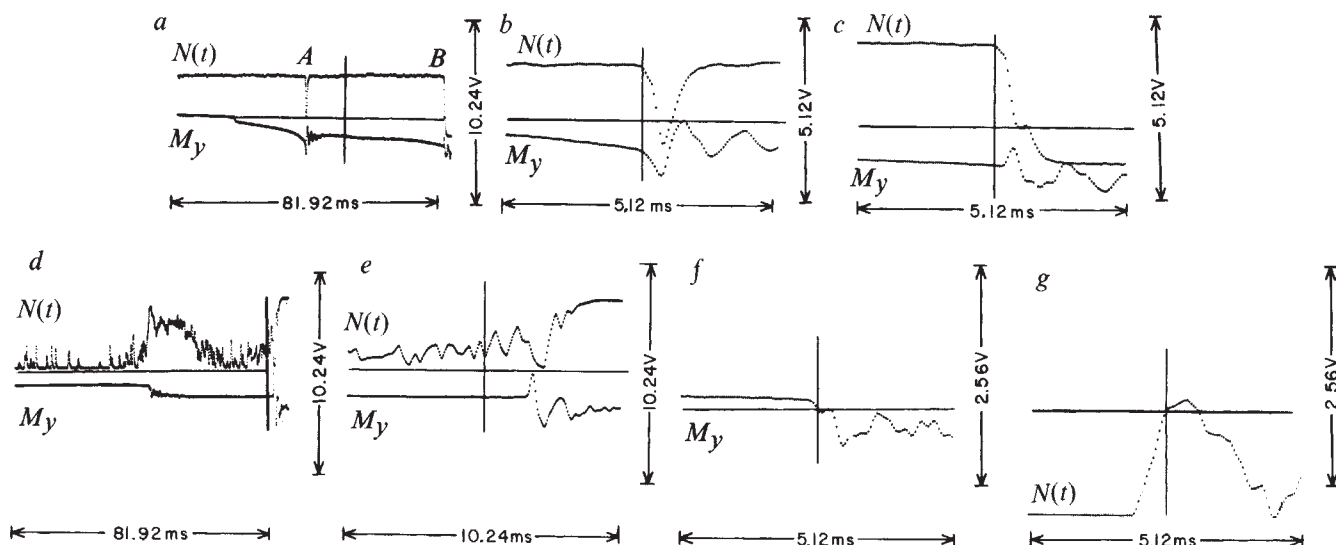


Fig. 2 Moment and load variation with time for Barre granite. *a*, BG-T14: vertical scale lengths for M_x and M_y are $8.65 \times 10^2 \text{ N m V}^{-1}$ and $1.03 \times 10^3 \text{ N m V}^{-1}$; *b*, BG-T29: vertical scale lengths for F and M_y are $9.08 \times 10^4 \text{ N V}^{-1}$ and $5.86 \times 10^2 \text{ N m V}^{-1}$, respectively; *c*, BG-T30: vertical scale length for F and M_y are $9.08 \times 10^4 \text{ N V}^{-1}$ and $5.86 \times 10^2 \text{ N m V}^{-1}$ respectively.

Fig. 3 Seismicity and tilt behaviour against time for Barre granite specimens T23 (*a-c*) and T24 (*d-g*). *b* and *c*, Specify tilt and seismicity for fractures *a* and *b*, respectively, of specimen T23. The conversion factor for M_y (lower trace) is $5.86 \times 10^2 \text{ N m V}^{-1}$. Vertical scale $N(t)$ (upper trace) for seismicity denotes $\log_{10} n(t)$ and represents the total emission count occurring within each 200- μs time window. The conversion factor for the total count is $\log_{10} n(t) = 10 V$, where V denotes voltage. Bandpass range is 0.3–1 MHz (*a-c*) and 1–2 MHz (*d-g*).



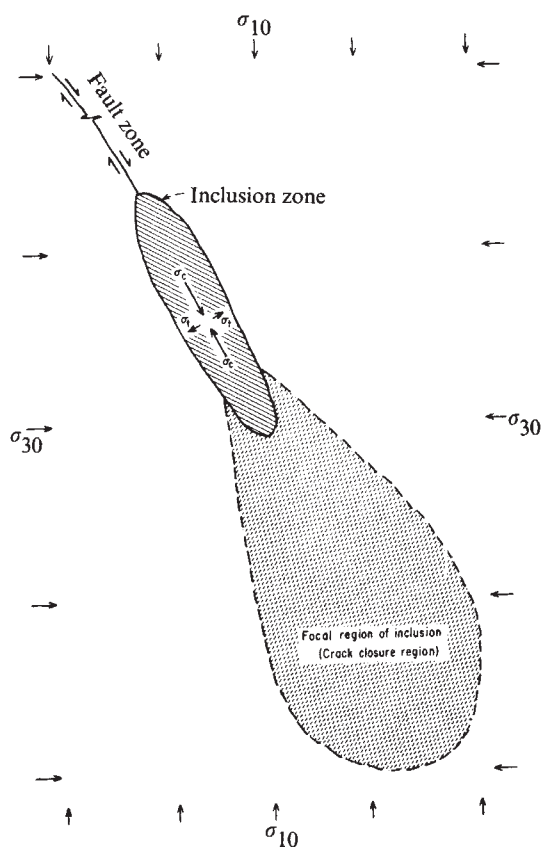


Fig. 4 Schematic illustration of fault zones, inclusion zone and focal region of the inclusion. Maximum and least principal stress applied at distances far removed from the inclusion boundary are indicated by σ_{10} and σ_{30} , respectively.

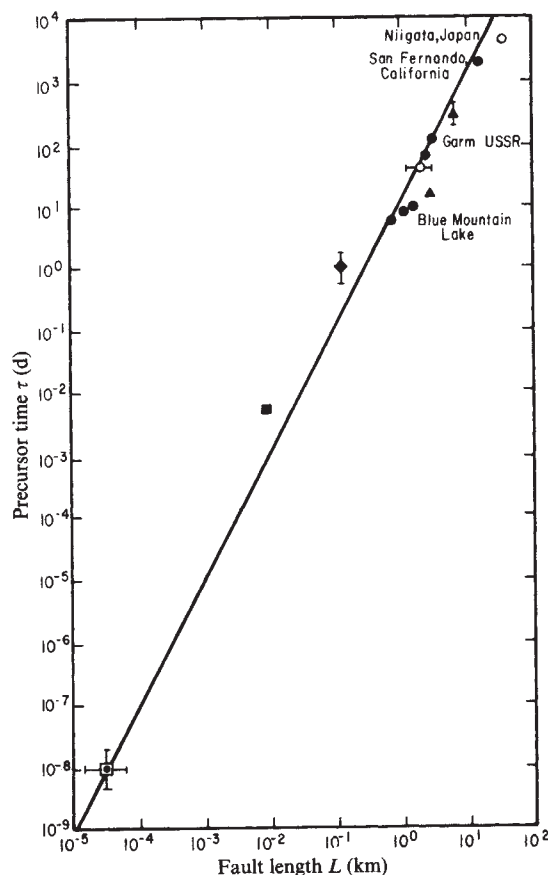


Fig. 5 Precursor time-fault length relationships for selected earthquakes, mine failures and laboratory failures. $\square$, Laboratory seismicity and tilt measurements; $\blacksquare$, noise count for a coal-mine rock fall; $\blacklozenge$, rock burst; $\bullet$, V_p/V_s anomaly; $\blacktriangle$, radon emission from Tashkent; $\circ$, crustal movements.

why the precursor time-magnitude-'fault' lengths relationships hold for both mine failures and earthquakes². Very briefly, the key hypothesis of this theory, termed the inclusion theory, is that the intense crack concentrations, experimentally observed to develop within localised regions in rock just before failure, can be mathematically modelled, and physically replaced by low modulus inclusions. The inclusions produce a threefold effect. First, the local principal stress axes rotate both within and outside the inclusion during the formation of the inclusion. Second, a state of tension stress is set up within and normal to the long axis of the inclusion, and third, the magnitude of the least principal stress increases in compression in the focal region of the inclusion, that is, the region into which the inclusion and its associated fault(s) will grow (Fig. 4). Thus, as the least principal stress increases, cracks (if present) in the focal region of the inclusion begin to close as the focal region begins to store strain energy, some of which will eventually be released as seismic radiation at the moment of failure. In this theory, the precursor time is defined to be the interval between the initiation of crack closure and failure, that is, the time between inclusion zone formation and the occurrence of catastrophic failure. The theory rests on the postulate that failure occurs when all cracks in the focal region of the inclusion close. At this point, the elastic contrast between the inclusion and its surroundings, the tension stress within the inclusion and the strain energy density throughout the focal region all attain their maximum possible values, and the process of catastrophic failure begins. Data in Figs 1, 2 and 3 support the hypothesis.

Anomalous tilt and seismicity behaviour before failure are predicted by the theory. For example, during the formation of the inclusion zone, tilting will be in a direction away from this zone. The tilt direction will reverse as cracks close in the focal

region. Seismicity within the focal region of the inclusion will decrease in response to the decrease in the principal stress difference in the focal region of the inclusion. The theory also predicts that the time duration of these anomalies is independent of rock type and is a function only of the size of the failure. For example, a time anomaly on the order of a few milliseconds will occur before failure in laboratory size (~ 1 – 10 cm length) specimens.

Figure 5 illustrates precursor time-'fault' length for selected earthquakes⁶, mine failures¹ and laboratory failures (based on the above experimental data). Fault length, assumed to be proportional to inclusion zone length for the laboratory specimens, was estimated to be in the range 1–10 cm. Note the time anomalies for the laboratory failures lie where predicted. Thus the scale-independent properties of the rock failure, at least within a precursor time interval of 10^{-8} – 10^4 d or, alternatively, seven orders of magnitude in 'fault' length, seem to have experimental justification. Tests on other rock types, such as oil shale, schist, marble and hydrostone, suggest tilt precursor anomalies also of the order of a few milliseconds. Further study is, however, required to test adequately the prediction that anomaly time is independent of rock type.

Dr S. Edelman of the US Department of Commerce, Bureau of Standards provided the piezoelectric transducers.

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¹ Brady, B. T., *Nature*, **252**, 549–552 (1974).

² Brady, B. T., *Pure appl. Geophys.*, **112**, 701–725 (1974).

³ Brady, B. T., *United States Bureau of Mines Report of Investigations* RI 8101, 9 (1976).

⁴ Brady, B. T., *Pure appl. Geophys.* (in the press).

⁵ Jaeger, J. C., and Cook, N., *Fundamentals of Rock Mechanics*, 513 (Methuen, London, 1969).

⁶ Whitcomb, J., Garmany, J., and Anderson, D., *Science*, **180**, 632–635 (1973).

Stable nuclear activation dependent on a protein synthesised during oogenesis

Ann Janice Brothers

Department of Zoology, Indiana University, Bloomington, Indiana 47401

A protein synthesised during oogenesis seems to be essential for the activation, during blastulation, of the nuclear genes essential for gastrulation and organogenesis. Nuclear transplantation experiments show that this interaction between the protein and the blastula nucleus produces a heritable state of nuclear activation.

It was established by the classical experimental embryologists¹⁻³ that the morphogenetic pattern of early development is already present in the cytoplasm of the fertilised egg. These morphogenetic substances are produced during oogenesis, stored in the egg cytoplasm and, after fertilisation, control the normal development of the zygote into the differentiated multicellular organism. The nature of these morphogenetic substances and the manner in which they function are unknown. Genes which exert maternal effects through modifications of the egg cytoplasm are therefore of special interest, since they provide a means of approaching the problem of how the egg cytoplasm acts in controlling early embryonic development⁴⁻⁸.

The o^+ substance

A simple recessive maternal effect gene (o for ova deficient), has been discovered in the Mexican axolotl (*Ambystoma mexicanum*). Females homozygous for o produce eggs which always arrest at gastrulation, regardless of whether or not the normal allele is introduced by the sperm at fertilisation. The genetic lesion occurs within the ovary and its expression is not affected by the maternal environment⁹. This suggests a gene-controlled modification of the egg cytoplasm affecting fundamental events in early morphogenesis. This modification is known to be in the nature of a cytoplasmic deficiency. The injection of nucleoplasm from a normal oocyte nucleus (germinal vesicle) or cytoplasm from a normal mature egg (after the germinal vesicle breaks down) into a mutant egg completely corrects the arrest at gastrulation¹⁰. The active component recovered from the oocytes of widely separated amphibian species seems to be functionally equivalent in the regulation of early embryonic development¹¹.

The normal allele of the o gene seems to direct the synthesis of a substance during oogenesis. This o^+ substance is present in the germinal vesicle by early "lampbrush" stage and its synthesis continues through most of oocyte growth. After the breakdown of the germinal vesicle the o^+ substance is found in the cytoplasm of mature eggs¹¹. The active factor cannot diffuse between the blastomeres¹⁰, and is no longer present in detectable amounts after the late blastula stage, suggesting that it has become fixed to some cell structure, rendering it unextractable, or that it has been degraded. The substance seems to be a protein(s) or to depend on protein(s) for its activity^{12,13}. Preliminary results indicate that it might be a high molecular weight acidic protein (G. M. Malacinski, personal communication).

The first differences between the mutant and normal eggs appear at mid-blastula stage. Before this, mutant and normal eggs exhibit essentially the same pattern of protein synthesis, as compared by double isotope acrylamide gel electrophoresis experiments. At the late blastula stage, however, eggs retain

the pattern of protein synthesis seen during earlier stages, whereas the normal eggs show an increased relative rate of synthesis of some proteins. This failure to alter or modulate the pattern of protein synthesis is also exhibited by mechanically enucleated amphibian eggs^{14,15}.

Amphibian development can proceed through blastulation without a functional genome; gastrulation, however, requires the presence of a nucleus^{3,16}. In *Xenopus* a spurt of new gene activity during mid-to-late blastula stages produces a population of new transcripts before gastrulation¹⁷. Normal axolotl eggs show transcription of various RNAs at mid-blastula stage. In contrast mutant blastulae show no incorporation of ³H-uridine autoradiographically¹⁸, but may display small amounts of incorporation by biochemical fraction analysis (R. Steele, A.J.B. and G. M. Malacinski, unpublished). These results suggest that transcription in the mutant blastula may be defective. An analysis of the pattern of RNA synthesis in both normal and mutant eggs is in progress. The data indicate that the o^+ substance is a protein(s) synthesised during oogenesis, stored in the ooplasm, and acting after fertilisation to control early developmental events. The presence of this protein(s) in the egg cytoplasm seems to be essential for the normal activation, during blastulation, of the nuclear genes required for gastrulation and organogenesis. A test of the stability of this nuclear activation is reported here.

Stable nuclear activation

A test can be obtained of the heritability of the nuclear activation by transplanting nuclei (which have been exposed to the o^+ substance) from various stages of normal blastulae into mutant eggs (which lack the o^+ substance). If the activation is not stable, then the recipient eggs, should in all cases develop like typical mutants—all arresting in gastrulation. If the activation is heritable, then the activated nuclei, but not the unactivated ones, should promote normal development of the

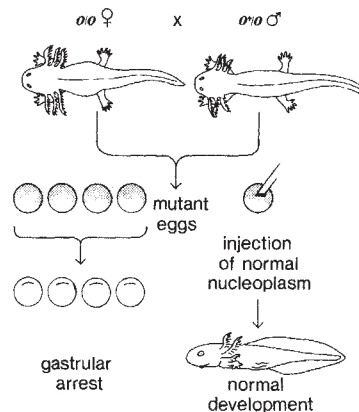


Fig. 1 A maternal effect mutation (o , for ova deficient) in the Mexican axolotl. Females homozygous for o produce eggs which always arrest at gastrulation. This gastrular arrest can be corrected by the injection of o^+ substance. This o^+ substance is produced during oogenesis under the direction of the normal allele of the o gene. The substance is found in the germinal vesicle and in the cytoplasm of normal mature eggs.

Table 1 Capacity of normal blastula nuclei to promote development of recipient mutant eggs

Developmental stage of the nuclear donor	Number (%) of embryos surviving to each stage						Feeding larva (1 month post-hatching)
	Total (100%)	Complete blastula	Early-gastrula (stage 10)	Neurula	Post-neurula	Larva	
Fertile mutant eggs—no transplant	888	883 (99%)	883 (99%)	0			
Early blastula (stages 8–8½)	234	130 (56%)	127 (54%)	0			
Mid-blastula (stage 8½)	87	41 (47%)	37 (42%)	25 (29%)	21 (24%)	18 (20%)	16 (18%)
Late mid-blastula (stage 8¾)	56	27 (48%)	24 (43%)	19 (34%)	16 (28%)	15 (27%)	13 (23%)
Late blastula (stage 9)	141	85 (60%)	69 (49%)	49 (35%)	46 (33%)	37 (25%)	28 (20%)
Late gastrula (stages 11½–12)	218	122 (56%)	75 (34%)	58 (27%)	43 (20%)	39 (18%)	25 (11%)
Control—transplants of nuclei from a normal blastula into an enucleated normal egg							
Early blastula (stage 8)	157	80 (51%)	75 (48%)	63 (40%)	54 (34%)	49 (31%)	37 (23%)
Late blastula (stage 9)	108	67 (62%)	66 (61%)	57 (53%)	48 (44%)	33 (30%)	29 (28%)
Mid-gastrula (stages 10½–11½)	98	56 (57%)	50 (51%)	36 (37%)	24 (24%)	22 (22%)	17 (17%)

A preliminary report of some of these results has been published in ref. 19. The nuclear transplants were done by an alteration (A. J. Brothers, unpublished) of the previously described techniques²⁰. The staging series used is a modification of the stage series described by Harrison²¹ and Carroll¹⁸. The modifications used in this paper are based on the developmental stage, measured by the time after first cleavage, at 18 °C. Stage 8 = 15–18 h. Stage 8½ = 18–20 h. Stage 8¾ = 20–22 h. Stage 9 = 22–24 h. Stage 9½ = 24–29 h. The genetic markers used for the nuclear transplants were those governing pigmentation (*d*), (*m*) and in some instances recessive mutations affecting early development (*f*), (*g*) and a cell autonomous lethal (*ur*) (refs 4, 13).

recipient enucleated mutant eggs. Transplantation of nuclei taken from normal early blastulae shows that those nuclei cannot improve the development of the recipient mutant eggs. Nuclei taken from normal mid-to-late blastulae are, however, capable of promoting normal development of the mutant eggs. (A preliminary report on the stability of late blastulae nuclei to support the development of recipient mutant eggs has been published¹⁹.) More complete results indicate that the stable alteration of the ability of the nucleus to support normal development is established at mid-blastulation. (Table 1).

Some of the animals derived from these transplants have been raised to sexually mature adults (Fig. 2). The spawnings from those animals developed normally and the genetic markers of the donor nuclei were expressed. A small amount of cytoplasm necessarily accompanies the transplanted nucleus. The contribution of this amount of cytoplasm to the correction is probably negligible, since at least 2% of the whole cytoplasm from a normal mature egg must be injected into a mutant egg in order to obtain correction¹⁰. Also, the active factor is no longer extractable from blastula stages, suggesting that it has become degraded or fixed to some cellular structure¹². Most important however, is the observation that normal nuclei from early blastulae do not show any capacity to alter the mutant phenotype when transplanted into mutant eggs, whereas normal late blastulae nuclei can support normal development.

Crude nuclear homogenates²² were made from normal late blastulae and aliquots were injected into fertile mutant and normal eggs. No correction of the phenotype of the mutant eggs were observed.

These observations suggest that the correction in the nuclear transplants is not fortuitously due to the accompanying cytoplasm nor is it due to some diffusible substance concentrated within the blastula nucleus. Therefore, the ability of normal late blastula nuclei to support development of the recipient mutant eggs must be a property of the intact nucleus itself.

Heritability of nuclear activation

A definitive test of the heritability of the activation was provided by serial clonal nuclear transplants of activated nuclei, using enucleated mutant eggs for recipients at each transplant generation (Fig. 3). The nuclei were transplanted into enucleated

mutant eggs, and those transplants were allowed to develop to early-blastulae (stage 8). At this point those nuclei had undergone at least nine mitotic divisions²² in the cytoplasmically deficient mutant eggs. Previous experiments had shown that nuclei from stage 8 blastulae had not yet exhibited activation, as measured by the capacity to promote normal development when transplanted into mutant eggs. Therefore, the serial clonal transplants would provide a test of whether or not a nucleus, once it has passed through the activation period, retains the capacity to support normal development through gastrulation and neurulation.

Some of the transplants in each series were taken at stage 8 to provide nuclear donors for the serial clonal transplants and a small portion of each nuclear donor blastula was also used for the determination of the autoradiographic pattern of ³H-uridine incorporation. The nuclei were again transplanted into enucleated mutant eggs, creating serial transplant clones. Members of each of those clones developed into normal larvae and showed the expression of the genetic markers of the original donor nuclei (Table 2). These experiments demonstrate that the nuclear activation (the capacity to support the normal development of early morphogenetic events) is a stable and heritable condition for more than 30 mitotic generations.

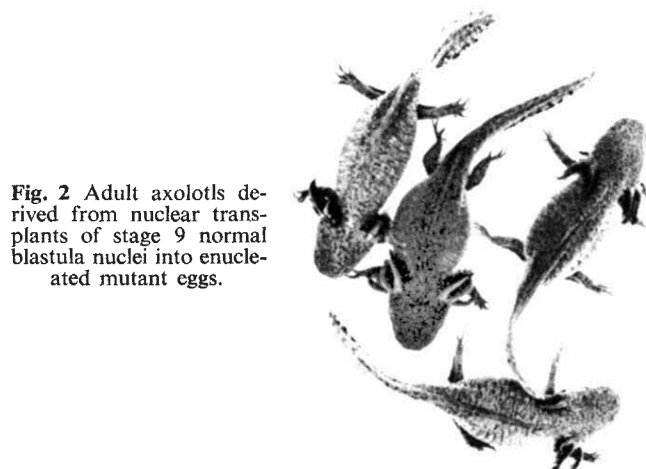


Fig. 2 Adult axolotls derived from nuclear transplants of stage 9 normal blastula nuclei into enucleated mutant eggs.

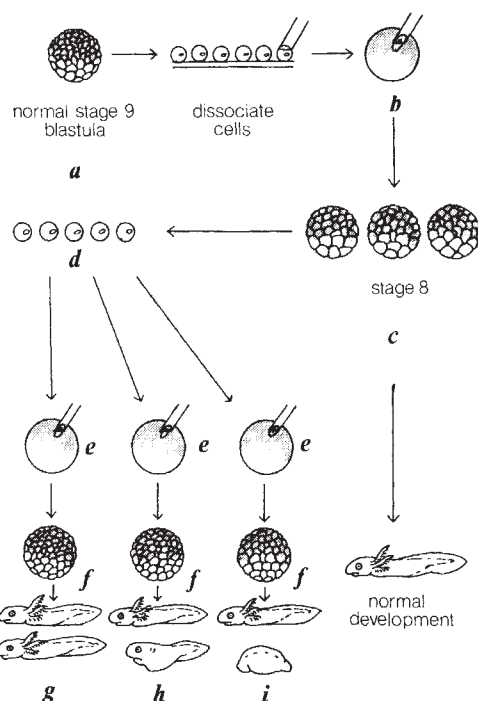


Fig. 3 Serial clonal nuclear transplants of activated nuclei using enucleated mutant eggs for recipients at each transplant generation. *a*, The nuclear donor is a normal stage 9, late blastula which shows intense ^3H -uridine incorporation in autoradiographs; *b*, the dissociated cells are used for nuclear transplants into an enucleated mutant egg; *c*, the nuclear transplants are allowed to develop to stage 8, early blastula stage. A few are sacrificed to serve as nuclear donors for the serial clonal transplants, and a portion of each nuclear donor blastula is taken for autoradiographic analysis. There is no detectable ^3H -uridine incorporation. *d*, The stage 8 nuclear donor blastula is dissociated in Ca^{2+} - and Mg^{2+} -free Steinberg's medium plus 0.005 M Na citrate, pH = 7.4 (ref. 48); *e*, serial clonal nuclear transplants are done, again using enucleated mutant recipient eggs; *f*, the serial clonal transplants are allowed to develop to stage 9, late blastula stage, where a few blastula from each serial clone are used for autoradiographic analysis. They show intense ^3H -uridine incorporation.

The capacity to express the nuclear genes necessary for gastrulation and organogenesis is stably altered, apparently independently of continued RNA synthesis (as measured by autoradiographic methods).

Normal early blastulae (stage 8) exhibit little or no nuclear incorporation of ^3H -uridine, but normal late blastulae (stage 9) show intense nuclear labelling. In contrast, mutant blastulae never show incorporation of ^3H -uridine autoradiographically¹⁸. Analysis of serial clonal transplants of normal late blastula nuclei into recipient mutant or normal eggs shows that the autoradiographic incorporation pattern of ^3H -uridine is the same as the pattern exhibited by control normal eggs.

In *Xenopus* rRNA, synthesis is not detectable until late cleavage or gastrula stages. Gurdon and Brown demonstrated that somatic cell nuclei which were synthesising rRNA, when transplanted into enucleated recipient eggs, did not continue synthesising rRNA in detectable amounts, but exhibited a pattern of rRNA synthesis typical of zygote nuclei²⁴. Cytoplasmic influence on the pattern of gene expression has also been described for somatic cell hybrids and heterokaryons^{25, 26}.

o^+ Substance interaction with nucleus

The ability of an embryonic nucleus to support development up through neural stages is dependent on an interaction with the o^+ substance. Injection of plasma containing o^+ substance into fertile mutant eggs enables them to develop normally, demonstrating that the nuclei of mutant eggs can respond to the o^+ substance¹⁰⁻¹³.

Transplantation of nuclei from mutant blastulae into normal enucleated recipient eggs tested the question of whether or not nuclei retain the capacity to interact with the o^+ substance throughout early development. As nuclei of mutant eggs undergo cleavage they are not exposed to the o^+ substance, since those eggs lack it. The transplantation of such nuclei into normal eggs, which contain the active factor, represents the first contact that those nuclei have with the o^+ substance. The ability of those nuclei to support normal development would provide evidence that they responded to the o^+ substance in the cytoplasm of the recipient normal eggs.

The experimental results (Table 3) demonstrate that late-cleavage (stage 7) and early-blastulae (stage 8½) mutant nuclei

Table 2 Serial clonal transplants of activated nuclei into mutant eggs

		No. of embryos surviving to each stage					
	Total	Complete blastula	Gastrula	Neurula	Post-neurula	Larva	Feeding larva
Series A							
Late blastula, normal stage 9 nucleus ($o^+/o^+, fg/fg^+$) put into a mutant egg	18	9*	6	6	6	5	4
Clone 1a	13	3	3	3	3	3	3
Clone 2a	18	5	5	5	3	1	0
Clone 3a	19	12	11	10	10	5	4
Series B							
Late blastula, normal stage 9 nucleus ($o^+/o^+, d/d, ut/ut^+$) put into a mutant egg	43	21†	15	13	10	7	6
Clone 1b	15	9‡	7	5	5	4	4
Clone 2b	17	10‡	6	4	2	1	1
Clone 3b	18	13‡	9	7	5	5	4
Clone 4b	13	9‡	6	5	2	2	1

*Three blastulae taken at stage 8 to serve as donors for nuclei for the clonal transplants (1a-3a) and for autoradiographic analysis of ^3H -uridine incorporation.

†Four blastulae taken at stage 8 to serve as donors for nuclei for the clonal transplants (1b-4b) and for autoradiographic analysis of ^3H -uridine incorporation.

‡A few stage 9 blastulae taken from each clone to check for autoradiographic incorporation of ^3H -uridine.

A preliminary report of the results obtained from series A serial clonal transplants has been published¹⁹.

Table 3 The capacity of the embryonic nucleus to interact with the o^+ substance in the egg cytoplasm

Developmental stage of the nuclear donor	Total (100%)	No. (%) of embryos surviving to each stage				Neurula	Larva
		Uncleaved	Abnormal	Normal	Partial		
Transplantation of a mutant blastula nucleus into an enucleated normal egg							
Late-cleavage (stage 7)	39	3 (8%)	11 (28%)	25 (64%)	8 (20%)	23 (59%)	18 (46%)
Early-blastula (stages 8-8½)	53	4 (8%)	4 (8%)	45 (84%)	6 (11%)	39 (73%)	29 (54%)
Late mid-blastula (stage 8½)	38	2 (5%)	6 (16%)	30 (79%)	18 (47%)	12 (31%)	0
Late-blastula (stage 9)	67	44 (66%)	23 (34%)	0	7 (10%)	0	0
Control—transplantation of a normal blastula nucleus into an enucleated normal egg							
Late-blastula (stage 9)	36	6 (17%)	5 (14%)	25 (69%)	4 (11%)	25 (69%)	19 (53%)

have the capacity to respond to the o^+ substance and that this response acts to alter the expression of the mutant phenotype. After advanced mid-blastula (by stage 8½) this capacity is lost; nuclei at this stage of development and older are no longer capable of supporting normal development if they have not been previously exposed to the o^+ substance. Mutant late blastulae (stage 9) show few or no mitoses, or incorporation of ^3H -thymidine¹⁸. Transplantation of those nuclei into normal eggs indicates that they are incapable of supporting normal cleavage. This suggests that late-blastula nuclei which have not been activated by an exposure to the o^+ substance are injured and are unable to function properly, even when exposed to normal cytoplasm.

Mode of action of o^+ substance

Cytoplasmic control of gene expression has been amply documented²⁴⁻²⁸. The migration of proteins between the cytoplasm and the nucleus has been reported for amoebae²⁹, insects³⁰⁻³¹ and amphibians³²⁻³⁴, as well as for many heterokaryons³⁵. Recent experiments have demonstrated the differential accumulation of certain classes of protein in nuclei^{31,36}. The association of proteins with DNA or the chromosomes, and the subsequent modification of DNA or chromosome behaviour has been described for various systems⁴¹⁻⁴³.

The o^+ substance seems to be a protein, which is apparently needed to establish the stable activation of the capacity to express the genes required to support normal development through gastrulation and organogenesis. It is tempting to speculate that the o^+ substance might be a regulatory protein, such as Davidson and Britten discussed in their model for eukaryotic gene regulation⁴⁴. The experimental results reported in this paper show that the nuclear activation is dependent on the presence of the o^+ substance within the cytoplasm of the fertilised egg. But these experiments do not show whether there is a direct interaction of the o^+ substance and the embryonic genome. Experiments to settle this issue are now in progress. In addition, the pattern of RNA synthesis in both normal and mutant eggs is currently under examination. It is expected that this approach will provide some insight into the possible relationship between the nuclear activation and gene expression.

Sonneborn's elegant experiments on the inheritance of mating type in *Paramecium* provide another demonstration of a cytoplasmically controlled stable nuclear determination. The sensitive period for this nuclear determination is restricted to one point in the nuclear "life cycle". During this period a cytoplasmic factor acts to produce a genetic alteration in the macronucleus (T. M. Sonneborn, personal communication). This is a stable alteration and is heritable through all subsequent clonal generations, even if the nucleus is later exposed to cytoplasm from the opposite mating type⁴⁵.

Cellular determination is endowed with a high degree of stability^{25,46}. Once it is established, the capacity to express a specific state of determination can be inherited over many cellular generations, even though the differentiated functions are not

expressed^{46,47}. The pattern of gene expression can be controlled and modified by cytoplasmic or extracellular factors^{25,27,41-43}.

In the axolotl the o^+ substance is produced during oogenesis, and the presence of this protein(s) in the egg cytoplasm is essential for the establishment of a stable nuclear activation. The sensitive period for this activation is restricted to one point during embryonic development. Apparently this alteration of the capacity to support development is the result of the activation of the nuclear genes required for gastrulation and neurulation. The isolation and characterisation of the o^+ substance provides an opportunity to examine the manner in which a morphogenetic substance functions.

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- Conklin, E. G., *Biol. Bull. Woods Hole*, **8**, 205-230 (1905).
- Wilson, E. B., *The Cell in Development and Heredity*, third ed. (Macmillan, New York, 1925).
- Davidson, E. H., *Gene Activity in Early Development* (Academic, New York, 1968).
- Briggs, R., *Ann. d'Embryol. Morphogen.*, **1**, 105-113 (1969).
- Garen, A., and Gehring, W., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2982-2985 (1972).
- Okada, M., Kleinman, J. A., and Schneiderman, H. A., *Dev. Biol.*, **37**, 55-62 (1974).
- Whittaker, J. R., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2096-2100 (1973).
- Illmensee, K., and Mahowald, A. P., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1016-1020 (1974).
- Humphrey, R. R., *Dev. Biol.*, **13**, 57-76 (1966).
- Briggs, R., and Cassens, G., *Proc. natn. Acad. Sci. U.S.A.*, **55**, 1103-1109 (1966).
- Briggs, R., *J. exp. Zool.*, **181**, 271-280 (1972).
- Briggs, R., and Justus, J. T., *J. exp. Zool.*, **167**, 105-116 (1968).
- Malacinski, G. M., and Brothers, A. J., *Science*, **184**, 1142-1147 (1974).
- Malacinski, G. M., *Dev. Biol.*, **26**, 442-451 (1971).
- Malacinski, G. M., *J. exp. Zool.*, **181**, 409-420 (1972).
- Gurdon, J. B., *The Control of Gene Expression in Animal Development* (Harvard University Press, Cambridge, 1974).
- Davidson, E. H., Crippa, M., and Mirsky, A. E., *Proc. natn. Acad. Sci. U.S.A.*, **60**, 152-129 (1968).
- Carroll, C. R., *J. exp. Zool.*, **187**, 409-422 (1974).
- Brothers, A. J., in *Proc. ICN-UCLA Conf. Dev. Biol.* (edit. by McMahon, D., and Fox, C. F.), (Benjamin, Reading, in the press).
- Briggs, R., Signoret, J., and Humphrey, R. R., *Dev. Biol.*, **10**, 233-246 (1964).
- Harrison, R. G., in *A Manual of Experimental Embryology*, (edit. by Hamburger, V.), 211-213 (University of Chicago Press, Chicago, 1966).
- Arms, K., *Dev. Biol.*, **26**, 497-502 (1971).
- Ignat'eva, G. M., *Ontogenez*, **6**, 626-629 (1972).
- Gurdon, J. B., and Brown, D. D., *J. molec. Biol.*, **12**, 27-35 (1965).
- Ephrussi, B., *Hybridization of Somatic Cells* (Princeton University Press, Princeton, 1972).
- Harris, H., Sidebottom, E., Grace, D. M., and Branwell, M. E., *J. Cell Sci.*, **4**, 499-525 (1969).
- Gurdon, J. B., and Woodland, H. R., *Biol. Rev. Camb. Phil. Soc.*, **42**, 233-267 (1968).
- Crippa, M., *Nature*, **227**, 1138-1140 (1970).
- Jelinek, W., and Goldstein, L., *J. Cell Physiol.*, **81**, 181-196 (1973).
- Kroeger, J., Jacob, J. J., and Sirlin, J. L., *Exp. Cell Res.*, **31**, 416-423 (1963).
- Paine, P. L., and Feldherr, C. M., *Exp. Cell Res.*, **74**, 81-98 (1972).
- Arms, K., *J. Embryol. exp. Morphol.*, **20**, 367-374 (1968).
- Meriam, R. W., *J. Cell Sci.*, **5**, 333-349 (1969).
- Di Berardino, M. A., and Hoffner, N. J., *Exp. Cell Res.*, **94**, 235-252 (1975).
- Apples, R., Bolund, L., and Ringertz, N. R., *J. molec. Biol.*, **87**, 339-355 (1974).
- Gurdon, J. B., *Nature*, **248**, 772-776 (1974).
- Bonner, W. M., *J. Cell Biol.*, **64**, 421-437 (1975).
- Hill, R. J., Maundrell, K., and Callan, H. G., *J. Cell Sci.*, **15**, 145-161 (1974).
- Sommerville, J., and Hill, R. J., *Nature new Biol.*, **245**, 104-106 (1973).
- Benbow, R. M., and Ford, C. C., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2437-2441 (1975).
- Barrett, R., Maryanka, D., Hamlyn, P. H., and Gould, H. J., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 5057-5061 (1974).
- Maniatis, R., Ptashne, M., and Maurer, R., *Nature*, **250**, 394-397 (1974).
- Davidson, E. H., and Britten, R. J., *Q. Rev. Biol.*, **48**, 565-612 (1973).
- Sonneborn, T. M., *Caryologia*, **6**, suppl., 307-325 (1954).
- Hadorn, E., *Dev. Biol.*, **13**, 424-509 (1966).
- Coon, H. G., *Proc. natn. Acad. Sci. U.S.A.*, **55**, 66-73 (1966).
- Steinberg, M., *Yb. Carnegie Instn Wash.*, **56**, 347 (1957).

Injected nuclei in frog oocytes provide a living cell system for the study of transcriptional control

J. B. Gurdon, E. M. De Robertis & G. Partington

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

Human HeLa nuclei injected into Xenopus oocytes synthesise RNA continuously for up to 1 month, and some of this RNA is translated into HeLa proteins. Nuclear proteins extracted from other cells enter the injected nuclei. This living cell system can be used for the study of transcriptional controls.

THE identification of chromosomal molecules that regulate gene transcription requires experimental systems in which the function of purified macromolecules can be tested efficiently. Cell-free systems which engage in transcription from isolated nuclei or chromatin are usually characterised by low efficiency (rate of transcription *in vitro* compared with that of the same nuclei *in vivo*), few new initiations (compared with *in vivo*), and sometimes inaccurate reading^{1,2} (choice of DNA strand and initiation or termination points). Even in the best systems involving isolated nuclei (for example, ref. 3), transcription has almost ceased within 2 h, and much of the labelled RNA obtained results from completion of chains initiated *in vivo*, rather than from new initiations *in vitro*. We have therefore explored the usefulness of living frog oocytes, which can be injected with nuclei and/or purified macromolecules as a system for investigating transcriptional controls. Earlier work has shown that *Xenopus* blastula nuclei continue to synthesise RNA for 3 d after injection into oocytes⁴. At the level of protein synthesis, reinitiation takes place on mRNA injected into oocytes for up to 2 weeks, as a result of which each molecule of mRNA may be translated up to 100,000 times⁵. We conclude from our results that human cultured cell nuclei injected into oocytes are transcriptionally active for up to 1 month, that some of their genes are expressed as newly synthesised proteins, and that they take up injected and endogenous proteins from the surrounding oocyte cytoplasm.

Persistence of injected nuclei in oocytes

The fate of ³H-thymidine (TdR)-labelled HeLa nuclei in *Xenopus* oocytes has been followed by autoradiography after fixing and sectioning. Oocytes of *Xenopus laevis* were prepared, injected and cultured as described previously^{4,6,7}, at 19 or 25 °C. In these conditions, oocytes appear morphologically normal for up to 1 month and injected nuclei remain intact. During the first few days after injection, nuclei undergo a substantial enlargement of eight times or more in volume, and their chromatin becomes dispersed (Figs 1*b* and 2*b*). Nuclei injected into the cytoplasm of an oocyte usually come to lie close to the oocyte nucleus (germinal vesicle) (Fig. 1*b*). With practice, it is possible to inject nuclei into the germinal vesicle (oocyte's nucleus), which is obscured in living oocytes by pigment and yolk; in most such experiments nuclei are deposited in an intact germinal vesicle or in the region where it breaks down. In these conditions, the injected nuclei can enlarge enormously (up to 100 times in volume), and may occupy the whole of the space inside the germinal vesicle whose chromosomes are displaced (Fig. 1*a*).

We have found that several different methods can be used

successfully to prepare nuclei for injection. In all cases the nuclei injected are surrounded by cytoplasm; however, for convenience, they are referred to here as nuclei. The method used for many of the experiments reported here is to suck cells into a small pipette so that the cell is ruptured, but cytoplasm not removed from around the nucleus—the method used to transplant nuclei to eggs⁶. It is essential however, not to prepare nuclei with the aid of detergents such as Triton X-100 or Nonidet P40; nuclei prepared in this way always die a few days after injection into oocytes. We usually inject about 200 HeLa nuclei into each oocyte.

Injected nuclei synthesise RNA for many days

Oocytes containing injected nuclei have been labelled by incubation in medium containing ³H-guanosine (³H-G) and ³H-uridine (³H-U) or by a second injection of ³H-guanosine triphosphate (³H-GTP). After the desired time, labelled oocytes are fixed, sectioned and autoradiographed. In all, oocytes tested for RNA synthesis (more than 200), nuclei injected into the cytoplasm or germinal vesicle were heavily labelled (Fig. 2*d*), whether the labelling period was as short as 30 min (after ³H-GTP injection), or as long as 36 h. Furthermore, nuclei seemed to synthesise RNA continuously throughout incubation. Even when oocytes containing HeLa nuclei were incubated in non-radioactive medium for 28 d at 19 °C and then in medium with ³H-U and ³H-G for a further 24 h, the injected nuclei were labelled strongly.

The amount of labelling over injected nuclei can be compared with that of the oocyte's germinal vesicle which serves as a convenient internal standard. For all labelling periods (0.5–24 h

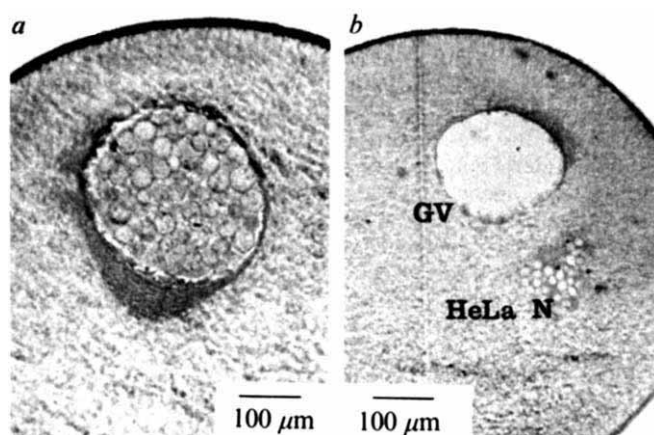


Fig. 1 *Xenopus* oocytes containing injected HeLa cell nuclei. GV, Germinal vesicle or nucleus of the oocyte; HeLa N, injected HeLa nuclei. *a*, GV filled with numerous enlarged HeLa nuclei; *b*, HeLa nuclei in the cytoplasm of an oocyte with an intact GV. Some of the nuclei in (*b*) are shown at a higher magnification in Fig. 2*b*. Oocytes were fixed 3 d after injection of nuclei. Cells used to provide nuclei were suspended in 0.25 M sucrose and 2 mM MgCl₂. HeLa cells were grown in plastic bottles by standard procedures, and checked for PPLO periodically.

at 1–28 d after injection), HeLa nuclei were more heavily labelled than the nucleoplasm of the germinal vesicle, but less so than the nucleoli in the germinal vesicle (Fig. 2c and d). It is also observed that greatly swollen nuclei (those injected 4 or more days previously) are about as heavily labelled, per area of section, as small, unswollen nuclei (those injected within the previous day). Nuclei therefore seem to increase their rate of RNA synthesis in proportion to their enlargement during incubation in oocytes. This effect is in striking contrast to the dilution of label observed during the enlargement of nuclei containing prelabelled DNA. Very rough estimates, based on the known rate of RNA synthesis by amphibian oocytes^{8,9}, indicate that the HeLa nuclei synthesise RNA at a rate which is at least the same in oocytes at 19 °C as in HeLa cells at 37 °C (refs 10 and 11). Sometimes a few of the injected nuclei fail to enlarge; after a few days they become pycnotic and cease to synthesise RNA.

The incorporation of ³H-U + ³H-G or of ³H-GTP by injected nuclei as just described is assumed to represent RNA synthesis, for the following reasons. The labelling of injected nuclei and of the oocyte germinal vesicle with its nucleoli is almost entirely eliminated by treatment with RNase before autoradiography. The germinal vesicles and especially nucleoli of *Xenopus* oocytes are known to be very active in RNA synthesis and inactive in DNA synthesis^{12–14}, and their pattern of labelling in these experiments was as previously observed. The possibility that label over injected nuclei represents the accumulation in them of RNA synthesised in, and transported from, the germinal vesicle is unlikely for two reasons. First, injected nuclei show the same level of labelling relative to the germinal vesicle after very short (30 min) or very long (36 h) labelling periods. Second, the injection of HeLa nuclei containing labelled RNA into unlabelled oocytes does not result in transfer of this RNA to the germinal vesicle, a fact which argues against a significant exchange of labelled RNA between nuclei in the same oocyte.

The results described so far indicate that HeLa nuclei remain transcriptionally active for at least 1 month. Since they seem to synthesise RNA at a substantial rate, we assume that extensive reinitiation of transcription takes place during this period.

Some HeLa genes are expressed in frog oocytes

Although HeLa nuclei actively synthesise RNA in frog oocytes, the results described so far do not show that this RNA includes mRNAs which code for any identifiable gene products. We have used two-dimensional isoelectric focusing—sodium dodecyl sulphate gel electrophoresis, the high resolution method of O'Farrell¹⁵—to detect HeLa-coded proteins in oocytes containing injected nuclei. Oocytes injected with HeLa nuclei were incubated in culture medium^{6,16} containing mixed ¹⁴C-amino acids. Proteins were extracted from the labelled oocytes, electrophoresed in two dimensions, and the resulting slab gels were dried and fluorographed^{17,18}.

Xenopus oocytes, labelled with a ¹⁴C-amino acid mixture, give a highly reproducible distribution of proteins (Fig. 3a). When oocytes with and without HeLa nuclei were labelled for 6 h immediately after injection, and their proteins were compared, no difference was observed. But when oocytes with and without HeLa nuclei were incubated for 3 d at 25 °C, labelled for 6 h and analysed by the same procedure, some extra proteins were seen in the nucleus-injected oocytes. One of these proteins, named 66/6.04 (because it has a molecular weight of 66,000, and an isoelectric point of 6.04), is strongly labelled (compare Fig. 3b and c). Proteins synthesised by HeLa cells include one with these properties, but mouse myeloma cells, and oocytes injected with myeloma nuclei (labelled after 3 d), do not (Fig. 3e). To characterise further this protein, region 66/6.04 was cut out from a two-dimensional gel of proteins synthesised by intact HeLa cells and from another gel of proteins from oocytes previously injected with HeLa nuclei. Material eluted from this region was treated with cyanogen bromide, and the resulting peptides were analysed by isoelectric focusing. The

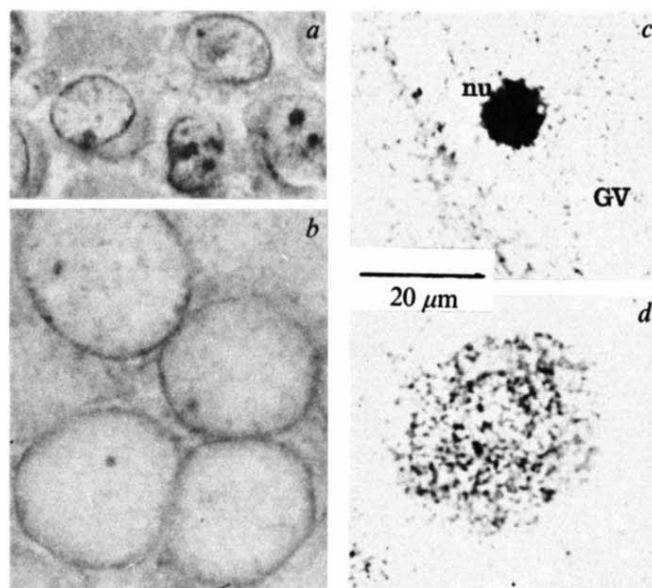


Fig. 2 HeLa nuclei in *Xenopus* oocyte cytoplasm. *a*, HeLa nuclei 5 min after injection; *b*, swollen HeLa nuclei 3 d after injection; *c*, autoradiograph of the germinal vesicle (GV) and a nucleolus (nu) of an oocyte; *d*, autoradiograph of a HeLa nucleus in oocyte cytoplasm. Both (*c*) and (*d*) show the same oocyte which, 3 d after injection of HeLa nuclei, was incubated in ³H-U and ³H-G for 24 h. In (*d*), the HeLa nucleus is more heavily labelled than the nucleoplasm of the oocyte but less heavily than the nucleolus (observed by grains).

peptides obtained from oocytes with HeLa nuclei and from intact HeLa cells are very well matched (Fig. 4); no detectable radioactivity was obtained from the labelled material extracted from region 66/6.04 of slab gels of uninjected oocytes. A labelled spot in position 66/6.04 has appeared in all eight independent two-dimensional analyses so far made of proteins from oocytes injected with HeLa nuclei 3 or more days previously, and in none of seven analyses of oocytes which did not contain HeLa nuclei. The spot appears whether nuclei are deposited in the cytoplasm or germinal vesicle of oocytes. We conclude that at least one HeLa protein is synthesised in oocytes injected with HeLa nuclei.

The HeLa nuclei injected into oocytes are, in effect, ruptured whole cells, and contain much of the original cells' cytoplasm. Protein 66/6.04 might therefore have been synthesised from cytoplasmic mRNA injected with the nuclei, but the following lines of evidence, although necessarily indirect, argue against this possibility and indicate that it is due to new transcription.

The new spot does not appear until about 3 d after injection, but any injected mRNA would be expected, from past experience^{5,7,19}, to be at least as active immediately after injection as at 3 d. This suggests that insufficient 66/6.04 message is carried over with the nuclei to be detected by two-dimensional electrophoresis during the first day after injection.

It could be argued that the observed time course might also arise if the injected nuclei were to contain mRNP particles or RNA precursors that would be translated only after processing inside the oocyte. To explore this possibility, we tested directly the amount of message carried over with our nuclear preparations. Oocytes were injected with RNA isolated from 4,000 HeLa cells prepared as for nuclear injection, that is, with 20 times more RNA than was carried in with the number of nuclei injected to give a clearly visible spot in position 66/6.04. The recovery of HeLa RNA was monitored by adding a known amount of globin mRNA to the HeLa cell extract, and obtaining a 60% recovery of its activity by translation^{7,19} in the injected oocytes. The RNA-injected oocytes showed no detectable synthesis of protein 66/6.04 in the course of 3 d, even though they had received, after allowing for recovery,

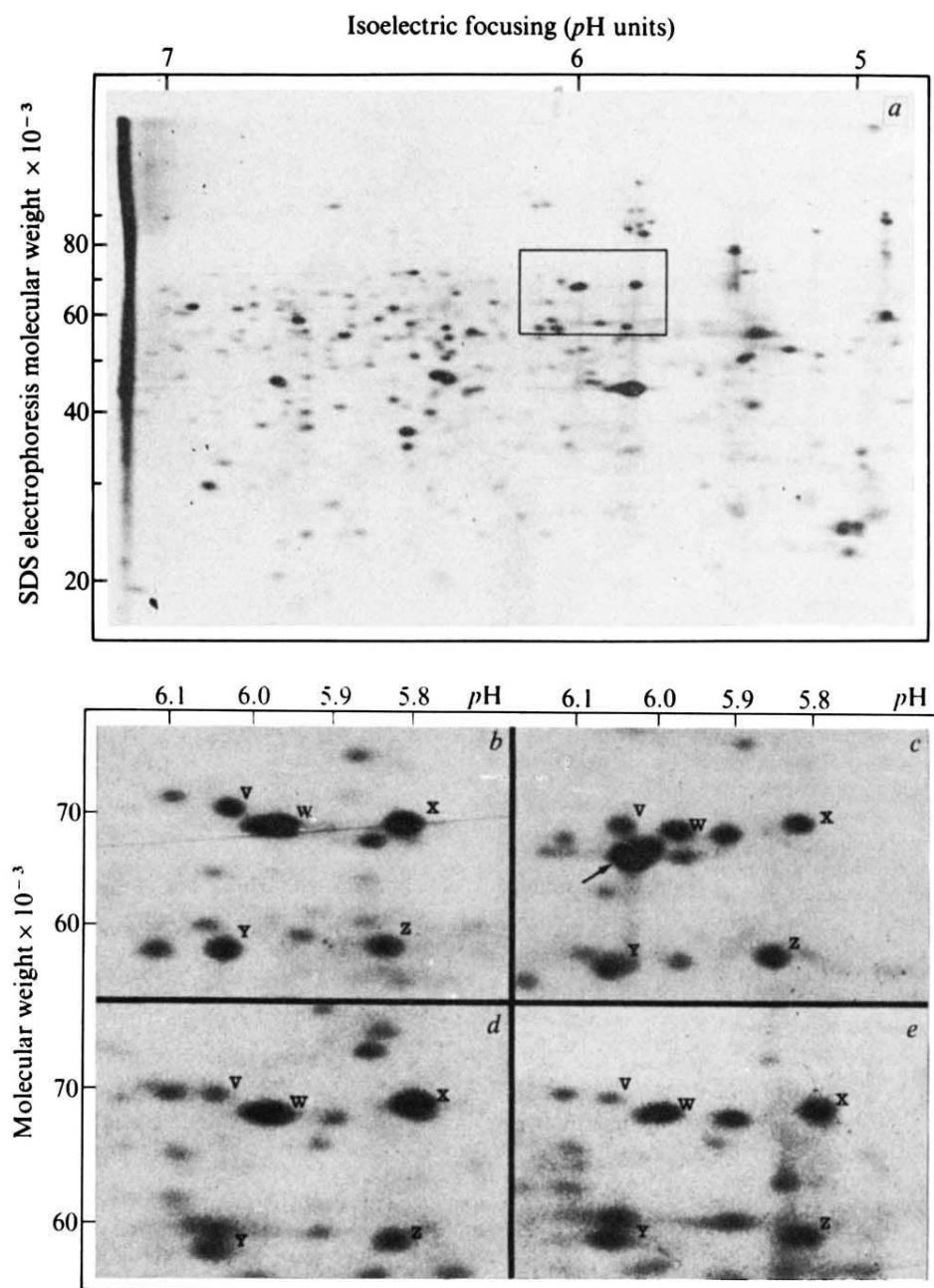


Fig. 3 Fluorographs of two-dimensional gels of *Xenopus laevis* oocyte proteins. Oocytes were labelled for 6 h in 2 μ l per oocyte of medium containing 250 μ Ci per ml of L-(14 C-U) amino acid mixture (57 mCi per atom), 3 d after injection of nuclei (25 $^{\circ}$ C). The oocytes were homogenised in 10 mM Tris-HCl, pH 7.4, 5 mM MgCl_2 , ribonuclease A 25 $\mu\text{g ml}^{-1}$ and deoxyribonuclease I 25 $\mu\text{g ml}^{-1}$. After standing for 10 min on ice, the samples were made 50 mM (a-c), or 100 mM (d and e) in NaCl and the yolk platelets were removed by centrifugation at 1,000g for 5 min. The isoelectric focusing dimension was performed as described before¹⁵ and electrophoresed for 18 h at 400 V. The second dimension was run in 15% polyacrylamide gels as described by Knowland³⁷. Some of the oocyte proteins are lettered from V to Z to help comparison. In addition to the proteins induced by the injected nuclei, the oocyte background shows minor variations due to differences in the extraction procedures (for example, the double spot at Y in d and e). a, Oocytes injected with saline. The rectangle indicates the area magnified in fluorographs b to e. b, Uninjected oocytes; c, oocytes injected with HeLa nuclei. The arrow indicates protein 66/6.04. d, Oocytes injected with HeLa nuclei and α -amanitin; e, oocytes injected with nuclei of a mouse myeloma cell line.

about 12 times more HeLa RNA than would be carried in with injected nuclei. In addition to this experiment, the α -amanitin experiment (below) and the fact that HeLa nuclei containing adenovirus genomes at the height of their transcriptional activity do not stimulate the synthesis of adenovirus proteins in oocytes (below), also argue strongly against the processing possibility.

If protein 66/6.04 is coded by RNA synthesised after the nuclei are injected into the oocyte, its synthesis might be expected to be sensitive to α -amanitin, an inhibitor of eukaryotic RNA polymerases²⁰. Oocytes were injected with HeLa nuclei, with or without α -amanitin, cultured for 3 d, and then labelled with 14 C-amino acids. The synthesis of protein 66/6.04 was eliminated in the α -amanitin-treated oocytes (Fig. 3d) but not in the controls. Enough α -amanitin was injected to give an intracellular concentration of 10 $\mu\text{g ml}^{-1}$, found previously to inhibit the synthesis of RNAs other than 28S, 18S and 4+5S in *Xenopus* oocytes²¹⁻²³. Several controls were performed in the same experiment. (1) Oocytes treated with α -amanitin as above showed a greatly reduced RNA synthesis over the germinal vesicle and injected nuclei, as judged by autoradio-

graphy. (2) The injected nuclei in α -amanitin-treated oocytes were morphologically normal. (3) To prove that the α -amanitin did not inhibit the translation of mRNA, purified rabbit globin mRNA was injected into some of the oocytes containing HeLa nuclei at the same time as α -amanitin, and found by one-dimensional gel analysis to be translated as efficiently as in other similar oocytes not injected with α -amanitin.

We conclude that protein 66/6.04 is synthesised in oocytes containing HeLa nuclei, by a coupled transcription-translation process. It seems that insufficient of the mRNA for this protein is injected with nuclei to give a labelled spot in position 66/6.04; this protein appears only when more mRNA has been synthesised by HeLa nuclei in oocytes in the course of about 3 d.

Gene expression by HeLa nuclei in oocytes is selective

Are all genes active in HeLa cells also expressed in oocytes containing HeLa nuclei? Several of the major proteins synthesised by HeLa cells are not distinguishable by two-dimensional analysis from major proteins synthesised by *Xenopus* oocytes, and the activity of these genes cannot therefore be

determined in these experiments. At least sixteen major proteins are distinguishable, however. Of these, two in addition to 66/6.04 are synthesised in oocytes 3 d after injection of HeLa nuclei, but we have not detected the synthesis of the other 13. Since most kinds of vertebrate mRNA are translated efficiently after injection into *Xenopus* oocytes (for example, refs 6, 24 and 25), we presume that many genes active in intact HeLa cells become inactive in oocytes, at the transcriptional or post-transcriptional (but pre-translational) levels.

To investigate the possibility that genes in injected nuclei may be transcribed but not translated, we have injected oocytes with HeLa nuclei prepared 6 or 12 h after infection with human adenovirus type 5 at a high multiplicity. RNA synthesised by these oocytes was labelled 3 d after nuclear injection, and hybridised to purified adenovirus 5 DNA in conditions of DNA excess. Over 10^6 c.p.m. of RNA was extracted from each sample of oocytes and about 50,000 c.p.m. was used for each hybridisation reaction. The same background level of hybridisation was observed with ^3H -RNA from oocytes containing infected nuclei as with ^3H -RNA from controls. Effective operation of the hybridisation methodology and recovery of RNA was demonstrated by extracting RNA from oocytes previously injected with ^3H -RNA from 18-h adenovirus-infected cells. A very low level of adenovirus transcripts would not have been detected by the procedures used. This result, however agrees with that of protein analyses. Oocytes containing 6-h or 12-h adenovirus-infected HeLa nuclei synthesise protein

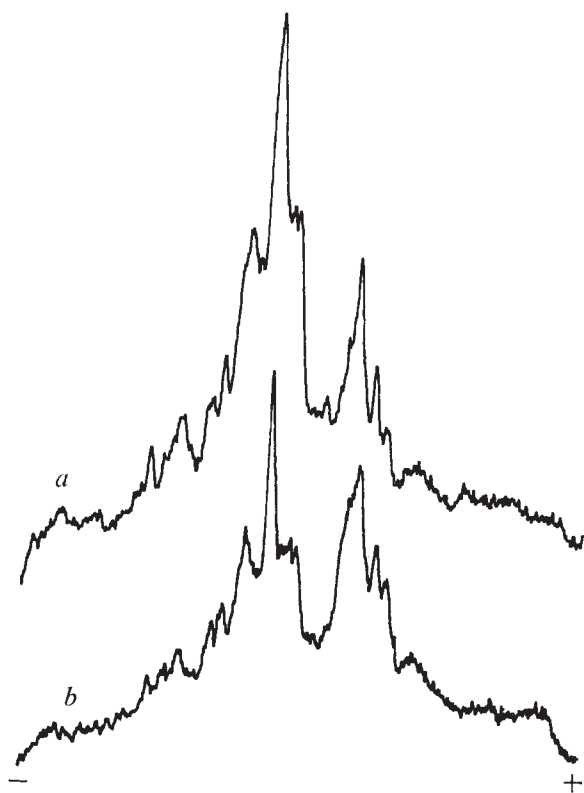


Fig. 4 Isoelectric focusing of cyanogen bromide peptides of protein 66/6.04. Total ^{14}C -proteins were electrophoresed in two-dimensional gels. After autoradiography the 66/6.04 region was cut out, homogenised and suspended in 70% formic acid containing cyanogen bromide ($200\text{ }\mu\text{g ml}^{-1}$). After shaking at 20°C for 12 h, the gel particles were centrifuged; the supernatant was lyophilised twice, dissolved in pyridine-acetate buffer, pH 6.5, incubated at 60°C overnight (to open the homoserine-lactone ring), and dried again. The samples were analysed by isoelectric focusing in a 4% polyacrylamide slab gel containing 2% ampholines (pH range 3.5–10) and 9 M urea. After fixing in 15% trichloroacetic acid, the gel was fluorographed and the film scanned in a Joyce-Loebl microdensitometer. *a*, Peptides from protein 66/6.04 of oocytes injected with HeLa nuclei; *b*, peptides from protein 66/6.04 of HeLa cells.

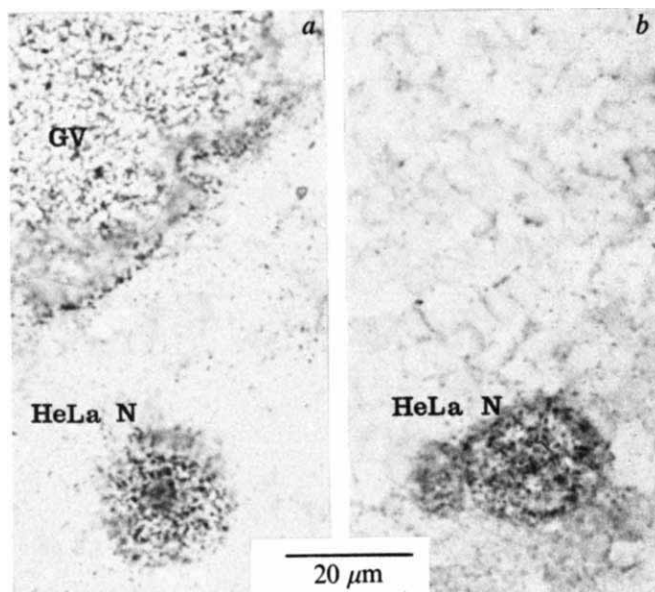


Fig. 5 *a*, Autoradiograph of an oocyte containing a HeLa nucleus. The oocyte was injected with HeLa nuclei; 5 d later it was injected with ^{125}I -labelled calf thymus histones (less H1) and incubated for a further 16 h before fixation. The HeLa nucleus (HeLa N) is about as heavily labelled as an equivalent area of the oocyte's nucleus (GV), and much more strongly labelled than the surrounding cytoplasm. *b*, Autoradiograph of a HeLa nucleus (and the edge of another one) in an oocyte; the oocyte was incubated in ^3H -leucine for 2 h and in unlabelled medium for a further 24 h, 4 d after it had been injected with HeLa nuclei. The nuclei are labelled much more strongly than the surrounding cytoplasm.

66/6.04, but do not synthesise two major adenovirus proteins (hexon and penton) which would have appeared in two-dimensional gel positions 115/6.10 and 70/7.00. Nor are the infected cell-specific polypeptides which would have been seen in positions 95/6.10, 78/6.00 and 65/6.25 synthesised by these oocytes. Apparently the adenovirus genome becomes transcriptionally inactive soon after infected nuclei are injected into oocytes, even though transcription by some HeLa genes in the same nuclei persists.

Uptake of proteins by injected HeLa nuclei in oocytes

If nuclei injected into oocytes are to serve as a test system for the function of macromolecules, it is essential to know that these molecules can gain access to the nuclear genes being studied. The following experiments show that some endogenous as well as introduced proteins rapidly enter HeLa nuclei from oocyte cytoplasm. Oocytes, injected with nuclei 4 d previously, were incubated in ^3H -leucine for 2 h, and then in excess unlabelled medium for 24 h. Autoradiography showed that the injected nuclei were strongly labelled well above the cytoplasmic background level (Fig. 5*b*). Since nuclei do not synthesise protein, they must have accumulated proteins synthesised in the cytoplasm; the fact that these proteins remained in the nuclei during a 24-h chase suggests that they may become associated with some intranuclear structures—possibly chromosomes. In another experiment, purified calf thymus histones labelled with ^{125}I (ref. 26) were injected into oocytes containing enlarged HeLa nuclei. The oocytes were fixed 24 h later, sectioned and autoradiographed. Both the injected nuclei and germinal vesicle were strongly labelled (Fig. 5*a*). The results of these experiments are supported by previous work: egg cytoplasmic proteins accumulate in endogenous or injected nuclei^{27–29}, whether or not puromycin is used to suppress protein synthesis, and ^{125}I -histones accumulate in oocyte germinal vesicles^{30,31}. Bonner³² has shown that non-histone proteins from an oocyte germinal vesicle selectively accumulate

in the germinal vesicles of other injected oocytes. We conclude from these results that nuclear proteins will enter injected nuclei in oocytes and therefore gain access to chromosomes whose genetic activity they may regulate.

Applications and prospects

The experimental system described can be used to test the gene-regulating activity of purified macromolecules which do not readily enter cells. High resolution two-dimensional gels and subsequent peptide analyses of separated proteins provide a sensitive test for changes in the activity of many different genes in the injected nuclei. Macromolecules whose effect is to be investigated can be injected into oocytes with the test nuclei. Oocytes may then be cultured for several days, so that the injected proteins may enter nuclei and associate with genes. Changes in gene activity can then be measured by subsequent labelling and analysis of newly synthesised RNAs or proteins.

It is very desirable to study changes in the activity of genes in injected nuclei, rather than changes in the activity of oocyte genes; this is because changes in the transcriptional activity of oocyte genes would be hard to detect over the very high background of proteins being synthesised from preformed, stable mRNA of which there is large stock in oocytes. The major advantages of this living cell system over cell-free systems with isolated nuclei are the very long persistence of transcription (for several weeks) and the more natural circumstances in which presumed regulatory molecules can function. The special advantage of frog oocytes over other living cell assay systems, such as injectable fibroblasts³³ or fused vesicles³⁴, is that sufficient label is incorporated by a few oocytes (importantly, a few injections) for a detailed biochemical analysis of newly synthesised RNAs or proteins. For each protein analysis described in this article, we have used material from five or less oocytes.

In addition to using *Xenopus* oocytes as a living test tube in which to test molecules regulating transcription, two directions in which this type of work may be fruitfully pursued are the following. One is to try to replace whole nuclei by chromatin or purified DNA, the efficient transcription of which might substantially increase the information obtainable from chromatin reconstitution experiments. The introduction of purified macromolecules into living frog oocytes and eggs has given encouraging results in preliminary investigations^{35,36}. The other is to develop a system in which inactive genes can be made

active in experimentally analysable conditions. This might be achieved by injecting into oocytes somatic nuclei from a tissue in which oocyte-active genes are repressed. The activation of such genes could be seen if nuclei from one strain or species were transplanted into oocytes of another in which the two-dimensional distribution of proteins is clearly different.

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- ¹ Roeder, R. G., Reeder, R. H., and Brown, D. D., *Cold Spring Harb. Symp. quant. Biol.*, **35**, 727–735 (1970).
- ² Reeder, R. H., and Brown, D. D., *J. molec. Biol.*, **51**, 361–377 (1970).
- ³ Marzluff, W. F., Murphy, E. C., and Huang, R. C., *Biochemistry*, **12**, 3440–3446 (1973).
- ⁴ Gurdon, J. B., *J. Embryol. exp. Morph.*, **20**, 401–414 (1968).
- ⁵ Gurdon, J. B., Lingrel, J. B., and Marbaix, G., *J. molec. Biol.*, **80**, 539–551 (1973).
- ⁶ Gurdon, J. B., *Control of Gene Expression in Animal Development* (Oxford and Harvard University Press, 1974).
- ⁷ Gurdon, J. B., Lane, C. D., Woodland, H. R., and Marbaix, G., *Nature*, **233**, 177–182 (1971).
- ⁸ La Marca, M. J., Smith, L. D., and Strobel, M. C., *Devl Biol.*, **34**, 106–118 (1973).
- ⁹ Scheer, U., *Devl Biol.*, **30**, 13–28 (1973).
- ¹⁰ Lin, H. J., and Chargaft, E., *Biochim. biophys. Acta*, **91**, 691–694 (1964).
- ¹¹ Stevens, R. H., and Amos, H., *J. Cell Biol.*, **50**, 818–829 (1971).
- ¹² Gall, J. G., and Callan, H. G., *Proc. natn. Acad. Sci. U.S.A.*, **48**, 562–570 (1962).
- ¹³ Davidson, E. H., Allfrey, V. G., and Mirsky, A. E., *Proc. natn. Acad. Sci. U.S.A.*, **52**, 501–508 (1964).
- ¹⁴ Gurdon, J. B., *Proc. natn. Acad. Sci. U.S.A.*, **58**, 545–552 (1967).
- ¹⁵ O'Farrell, P. H., *J. biol. Chem.*, **250**, 4007–4021 (1975).
- ¹⁶ Colman, A., *J. Embryol. exp. Morph.*, **32**, 515–532 (1974).
- ¹⁷ Bonner, W. M., and Laskey, R. A., *Eur. J. Biochem.*, **46**, 83–88 (1974).
- ¹⁸ Laskey, R. A., and Mills, A. D., *Eur. J. Biochem.*, **56**, 335–341 (1975).
- ¹⁹ Lane, C. D., Marbaix, G., and Gurdon, J. B., *J. molec. Biol.*, **61**, 73–91 (1971).
- ²⁰ Stirpe, F., and Fiume, L., *Biochem. J.*, **105**, 779–782 (1967).
- ²¹ Roeder, R. G., *J. biol. Chem.*, **249**, 241–248 (1974).
- ²² Tochini-Valentini, G. P., and Crippa, M., *Cold Spring Harb. Symp. quant. Biol.*, **35**, 737–742 (1970).
- ²³ Colman, A., *Eur. J. Biochem.*, **57**, 85–96 (1975).
- ²⁴ Vassart, G., Refetoff, S., Brocas, H., Dinsart, C., and Dumont, J. E., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 3839–43 (1975).
- ²⁵ Lanclos, K. D., and Hamilton, T., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 3934–3938 (1975).
- ²⁶ Greenwood, F. C., Hunter, W. M., and Glover, J. S., *Biochem. J.*, **89**, 114–123 (1963).
- ²⁷ Arms, K., *J. Embryol. exp. Morph.*, **20**, 367–374 (1968).
- ²⁸ Merriam, R. W., *J. Cell Sci.*, **5**, 333–349 (1969).
- ²⁹ Eckert, R. E., and Smith, L. D., *Devl Biol.*, **24**, 559–576 (1971).
- ³⁰ Gurdon, J. B., *Proc. R. Soc. B*, **176**, 303–314 (1970).
- ³¹ Bonner, W. M., *J. Cell Biol.*, **64**, 421–430 (1975).
- ³² Bonner, W. M., *J. Cell Biol.*, **64**, 431–437 (1975).
- ³³ Grässmann, A., and Grässmann, M., *Z. physiol. Chem.*, **352**, 527–532 (1971).
- ³⁴ Papahadjopoulos, D., Poste, G., and Mayhew, E., *Biochim. biophys. Acta*, **363**, 404–418 (1974).
- ³⁵ Gurdon, J. B., *Nature*, **248**, 772–776 (1974).
- ³⁶ Gurdon, J. B., and Brown, D. D., *Symposium on Molecular Biology of the Genetic Apparatus—its relation to Cancer, Aging, and Medical Genetics* (edit. by T'so, P.) (North-Holland, Amsterdam, in the press).
- ³⁷ Knowland, J. S., *Genetics*, **78**, 383–394 (1974).

Developmental shifts in the synthesis of heterogeneous nuclear RNA classes in the sea urchin embryo

Lewis M. Dubroff* & Martin Nemer

Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111

Three molecular classes with different properties can be distinguished in the heterogeneous nuclear RNA (HnRNA) of sea urchin embryos. The relative proportions of these classes, which we have previously designated α , β and γ HnRNA, undergo marked changes during development from morula to early gastrula. Such changes suggest the existence of more than a single HnRNA function, as well as specific roles for different HnRNA classes in embryonic development.

*Present address: Mayo Clinic, Rochester, Minnesota 55901.

THE functions of heterogeneous nuclear RNA (HnRNA) may well be elucidated by investigating the involvement of HnRNA in embryonic development. Such an approach is made promising by our demonstration that HnRNA in the sea urchin embryo is not a uniform class of molecules, but is comprised of distinct molecular types¹. In the study reported here we asked whether development is characterised by shifts in the synthesis of these distinct molecular types of HnRNA.

Early sea urchin development involves (1) the rearrangement of the cells of the morula to form the hollow sphere of the early blastula; (2) rotation of the ciliated blastula inside its ferti-

lisation membrane; (3) lysis of the membrane to yield a hatched blastula; (4) migration of mesenchyme cells into the blastocoel, and (5) invagination to produce a gastrula. The early stages (1–3) do not seem to depend on the transcription of the embryonic genome, in so far as development to the blastula stages can proceed normally when RNA synthesis is blocked². In spite of the appearance of newly synthesised mRNA in polyribosomes^{3,4}, the proteins synthesised during this period are minimally different from those made in the absence of RNA synthesis, that is, exclusively from maternal RNA templates⁵. This apparent absence of an immediate influence of morula and blastula transcription does not exclude an effect on later morphogenesis. Indeed, there are intriguing critical periods during early development, in which a transient block on transcription^{6,7} exerts a morphogenetic influence only later,

parations to poly(U), in so far as the amount bound would be reduced by the cleavage of portions of the molecule from oligo(A)- or poly(A)-containing HnRNA segments. Early blastulae and mesenchyme blastulae with different isotope labels were lysed, portions of the lysates were mixed, and the poly(U) binding of HnRNA mixtures was compared with that of the HnRNAs prepared from the original lysates (experiment 1, Table 1). The percentages of poly(U) filter-bound HnRNA from individually prepared HnRNAs clearly differed for the two stages, 20–25% for the early blastula and 37–39% for the mesenchyme blastula. We conclude that the lower value for the earlier stage is not attributable to the presence of miscible degradative agents, because the value of the mesenchyme blastula HnRNA was not significantly reduced as a result of having been prepared from a mixed lysate.

Table 1 Comparison of the percentage of molecules binding to poly(U) in HnRNA prepared from mixed and unmixed lysates of embryos at various stages

Developmental stage	Experiment 1		Poly(U) filter binding Experiment 2		Experiment 3	
	Early blastula ³ H	Mesenchyme blastula ¹⁴ C	Morula ³ H	Mesenchyme blastula ¹⁴ C	Morula ³ H	Mesenchyme blastula ¹⁴ C
Method of preparation						
Mixed lysate	25	37	40	36	22	28
Individually prepared	20	39	40	36	27	39

Early blastula and morula embryos of *Lytechinus pictus* were labelled for 60 min with ³H-uridine, and mesenchyme blastula embryos were labelled for 60 min with ¹⁴C-uridine. HnRNA was prepared from a portion of each batch of embryos. Immediately after lysis of embryos, lysates of early blastula and mesenchyme blastula embryos, and morula and mesenchyme blastula embryos were mixed in 5:1 portions in each case. Nuclei and HnRNA were then prepared from each of these mixed lysates. Preparation of intact nuclei from embryos at different stages of development requires different lysis conditions because of large changes in nuclear volume during early embryogenesis¹⁸. Embryos were washed with 1 M dextrose, suspended in 30–60 volumes of 2 mM MgCl₂, and then passed through a hypodermic needle¹, except that an 18-gauge needle was used for morula (4 h), 20 for early blastula (7 h), 21 for mid-blastula (10 h), 22 for rotating blastula (13 h), and 23 for hatched blastula (16 h) and mesenchyme blastula (21 h). Nuclei were purified as before¹. Electron microscopy revealed that some ribosomes remained attached to these nuclei. A small amount of rRNA of apparent cytoplasmic origin could also be detected when HnRNA preparations were sedimented. This rRNA can be eliminated by pelleting nuclei in a solution containing 0.005 M EDTA in addition to the 1% Triton X-100 solution previously described¹. Preparation of HnRNA and the method of binding to poly(U) filters have also been described¹. RNA was assayed as uridine incorporation in alkaline-labile, acid-precipitable material.

just before or after gastrulation. Thus early stage RNA transcripts may be more important for later development than for early stage protein synthesis. Our fractionation of HnRNA into molecular types may prove useful for analysing the nature and significance of these early RNA transcripts.

The molecular classes that we have characterised and quantified in the sea urchin embryo¹ have been defined as follows: (1) α HnRNA contains internal oligo(A)₂₅ and oligo(A)₁₂ stretches, (2) β HnRNA contains a 3' terminal poly(A)₁₇₅ segment and internal oligo(A)₁₂ stretches and (3) γ HnRNA lacks both poly(A)₁₇₅ and oligo(A)₂₅. These molecular classes have characteristic mean S values of 37, 30 and 36S, respectively, and are apparently not artefactually derived from larger molecules. The study reported here shows that the β HnRNA, which probably bears a precursor relationship to polyadenylated mRNA, is not synthesised in constant proportion as development proceeds. This observation contrasts with the constant amount of polyadenylated HnRNA in resting and growing fibroblast cells⁸. Indeed, the relative rates of synthesis of all three molecular types undergo pronounced changes as a function of embryonic development. Such compositional changes in HnRNA reinforce our concept that HnRNA molecules, in addition to differing in specific nucleotide sequences, fall into classes which are very likely responsible for separate physiological functions.

Integrity of HnRNA from early stages

Unambiguous measurements of changes in the percentages of α , β and γ HnRNA during early development depend on an assurance of the integrity of HnRNA extracted at each embryonic stage. Mixing experiments were performed to test for the absence of miscible (soluble) degradative agents. The integrity of HnRNA can be monitored by binding mixed pre-

This mixing protocol can be used to choose between divergent values, such as the 40 and 22% (experiments 2 and 3, Table 1) for two preparations of morula HnRNA. The lower value can probably be attributed to the action of degradative agents, since mixing the low binding morula HnRNA with mesenchyme blastula HnRNA reduces the binding value of the latter from 39 to 28% (experiment 3). In contrast, the morula HnRNA of high value (experiment 2) does not affect the mesenchyme blastula HnRNA value. The poly(U) binding of morula HnRNA is then approximately equal to that of mesenchyme blastula HnRNA. In our conditions of preparation, enzymes able to degrade HnRNA in exogenous nuclei may in certain cases be active at the morula stage but not at the early blastula stage or mesenchyme blastula stage. The integrities of HnRNAs were also compared by sedimenting total HnRNA in denaturing solvent⁹. Slightly higher S values were obtained for HnRNA of 13-h rotating blastulae (34–36S) compared with 20-h mesenchyme blastulae (28–30S). The lower mean S value for total HnRNA in the later stage embryo may be attributed to an increase in the proportion of β HnRNA, which possesses the smallest mean S value of the three HnRNA classes¹.

Developmental changes in HnRNA composition

The HnRNA which binds to poly(U) filters consists of at least two general classes, α HnRNA containing oligo(A) and β HnRNA containing poly(A)¹. These components can be resolved by elution with a gradient of logarithmically increasing concentration of the denaturing solvent formamide and decreasing salt concentration, the α HnRNA being eluted at 15% and the β HnRNA at 40% formamide. These observations were made with HnRNA from 13-h prehatched, rotating blastulae¹, and the elution profile for this stage has been re-

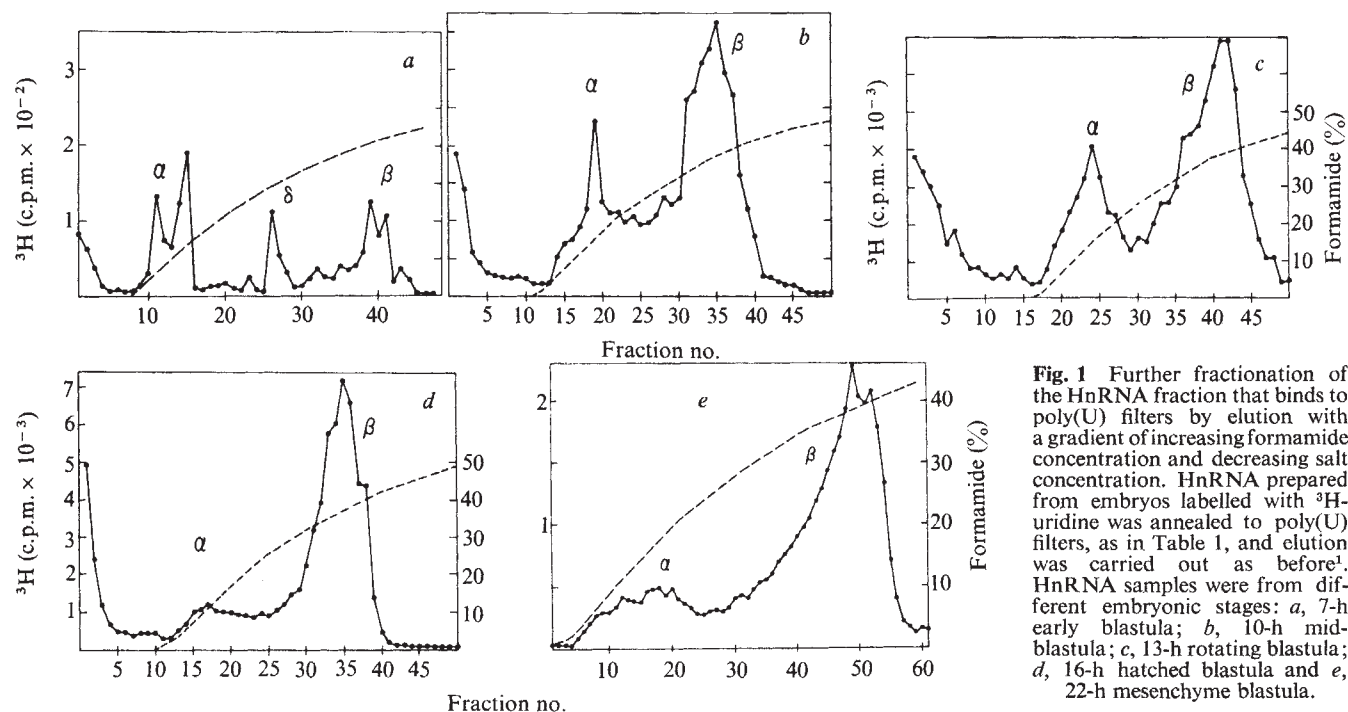


Fig. 1 Further fractionation of the HnRNA fraction that binds to poly(U) filters by elution with a gradient of increasing formamide concentration and decreasing salt concentration. HnRNA prepared from embryos labelled with ^3H -uridine was annealed to poly(U) filters, as in Table 1, and elution was carried out as before¹. HnRNA samples were from different embryonic stages: *a*, 7-h early blastula; *b*, 10-h mid-blastula; *c*, 13-h rotating blastula; *d*, 16-h hatched blastula and *e*, 22-h mesenchyme blastula.

produced in Fig. 1c. Here and in earlier stages (Fig. 1a and b) the α HnRNA represents a substantial proportion, at least 30%, of the labelled HnRNA bound to poly(U). In later blastula stages (Fig. 1d and e) the proportion of α HnRNA is considerably diminished, with the β HnRNA very much predominant. The ratio of β HnRNA to α HnRNA increases some 2–4-fold during development from early to late blastula stages. Details of the elution profiles reveal that the α HnRNA tends to be resolved into two peaks. Another peak, eluted at 30% formamide, is especially evident in the 7-h early blastula (δ , Fig. 1a). The component can be made to bind partially (~50%) to oligo(dT) cellulose⁹. This behaviour resembles neither that of α HnRNA, which does not bind to oligo(dT), nor that of β HnRNA, which binds completely to oligo(dT)¹. Further characterisation of the δ component is in progress (unpublished results of L.M.D.)

We have shown previously that the α , β and γ classes of HnRNA could be separated and quantitated by virtue of their

binding properties to oligo(dT)–cellulose and poly(U) filters¹. β HnRNA binds oligo(dT)–cellulose through its poly(A) segment. α HnRNA binds through oligo(A) segments to poly(U), together with β HnRNA; however, it does not bind to oligo(dT)–cellulose. Thus the percentage of α HnRNA is obtained by subtracting the percentage bound to oligo(dT)–cellulose (β HnRNA) from the percentage bound to poly(U) filters ($\alpha + \beta$ HnRNA). γ HnRNA will not bind to poly(U) filters.

Analyses of HnRNA preparations from early blastula (7 h), rotating blastula (13 h) and mesenchyme blastula (21 h) embryos exemplify the measurements of differential binding (Table 2). The HnRNA from each stage was separated into fractions bound and not bound to oligo(dT)–cellulose, and these fractions were further examined for bindability to oligo(dT)–cellulose or poly(U) filters (Table 2). For each of the embryonic stages the bound fraction (11%, 16% and 33% for the respective stages above) was almost completely retained on

Table 2 Binding measurements of RNA and oligo(dT)–cellulose-fractionated RNA to oligo(dT)–cellulose and poly(U) filters

RNA tested	% Total HnRNA bound to oligo(dT)–cellulose	% Total HnRNA bound to poly(U) filter	% Not bound
HnRNA from 7-h early blastula	11	24	76 (γ HnRNA)
Rerun HnRNA bound to oligo(dT)	10 (β HnRNA)	10	
Rerun HnRNA not bound to oligo(dT)	<0.1	14 (α HnRNA)	
Sum of rerun HnRNA		24	
HnRNA from 13-h blastula	16	36	64 (γ HnRNA)
Rerun HnRNA bound to oligo(dT)	16 (β HnRNA)	15	
Rerun HnRNA not bound to oligo(dT)	<0.1	15 (α HnRNA)	
Sum of rerun HnRNA		30	
HnRNA from 21-h mesenchyme blastula	33	37	63 (γ HnRNA)
Rerun HnRNA bound to oligo(dT)	32 (β HnRNA)	31	
Rerun HnRNA not bound to oligo(dT)	<0.1	5 (α HnRNA)	
Sum of rerun HnRNA		36	

HnRNA was from embryos labelled for 60 min with ^3H -uridine. Assays of binding to poly(U) filters and oligo(dT)–cellulose have been described previously¹. RNA was assayed as uridine incorporation in alkaline-labile, acid-precipitable material in all fractions. See text for definitions of α , β and γ HnRNA. The 14% in α and the 11% in β HnRNA in the 7-h early blastula must be corrected for the approximately 3% in the δ component, estimated from Fig. 1a. Since approximately half of the δ component binds to oligo(dT) together with β HnRNA and the other half is not bound, in company with α HnRNA (unpublished results of L.M.D.), the corrected values are approximately 12% in α HnRNA and approximately 9% in β HnRNA.

a subsequent passage through oligo(dT)-cellulose or poly(U) filters, and the unbound fraction remained unbound after a second passage through oligo(dT)-cellulose. Repassage of the unbound fraction through poly(U) filters, however, resulted in the binding of part of this fraction to an extent representing 14%, 15% and 5% of the respective total HnRNAs. At each stage this additional HnRNA plus the HnRNA bound to oligo(dT)-cellulose, was approximately equivalent to the percentage of the total HnRNA bound originally by poly(U) filters. Therefore, HnRNA that is bindable to poly(U) filters comprises two classes at each stage, distinguishable by their binding properties to oligo(dT)-cellulose. Quantitative differences are observed in the content of α , β and γ HnRNA classes in the 7-h morula, 13-h rotating blastula and 21-h mesenchyme blastula as follows: α HnRNA comprises 12%, 15% and 5% at these successive stages, β HnRNA is 9%, 16% and 33% and γ HnRNA represents 76%, 64% and 63%, respectively.

The quantitative changes in HnRNA composition, particularly in the relative incorporations in α and β HnRNA, can be evaluated by two methods, either graphically estimating components eluted by formamide from poly(U), as in Fig. 1, or the differential binding to oligo(dT)-cellulose and poly(U) filters, as exemplified by Table 2. Measurements can be made easily on a multitude of samples by the method of differential binding. The percentages of labelled HnRNA bound to oligo(dT)-cellulose and poly(U) filters are recorded in Fig. 2 for numerous batches of embryos from the 4-h morula to the 22-h mesenchyme blastula stage. The amounts of HnRNA bound to these two polymers differ widely from each other and fluctuate differently during development. The fraction bound to oligo(dT)-cellulose is a direct measure of the β HnRNA, whereas the difference function between these two curves represents α HnRNA. The γ HnRNA fraction is not bound to poly(U) filters, and thus its relative incorporation during development is represented by the inverse of the curve for binding to poly(U) filters. Continuous curves for each of these HnRNA components, drawn from these data are given in Fig. 3. They seem to demonstrate three periods during early embryogenesis, each characterised by a distinctively different

Fig. 2 Labelled HnRNA from various stages of development bindable to oligo(dT)-cellulose columns and poly(U) filters. Embryos at various stages of development were labelled with $10\text{--}100\ \mu\text{Ci ml}^{-1}$ of ^3H -uridine for 60 min. HnRNA prepared from these embryos was bound to oligo(dT)-cellulose (O) or poly(U) filters (●). Each symbol represents the mean and standard deviation derived from determinations on 5 to 18 batches of embryos for each stage. The points represent the average stage at which the 60 min incubations were terminated.

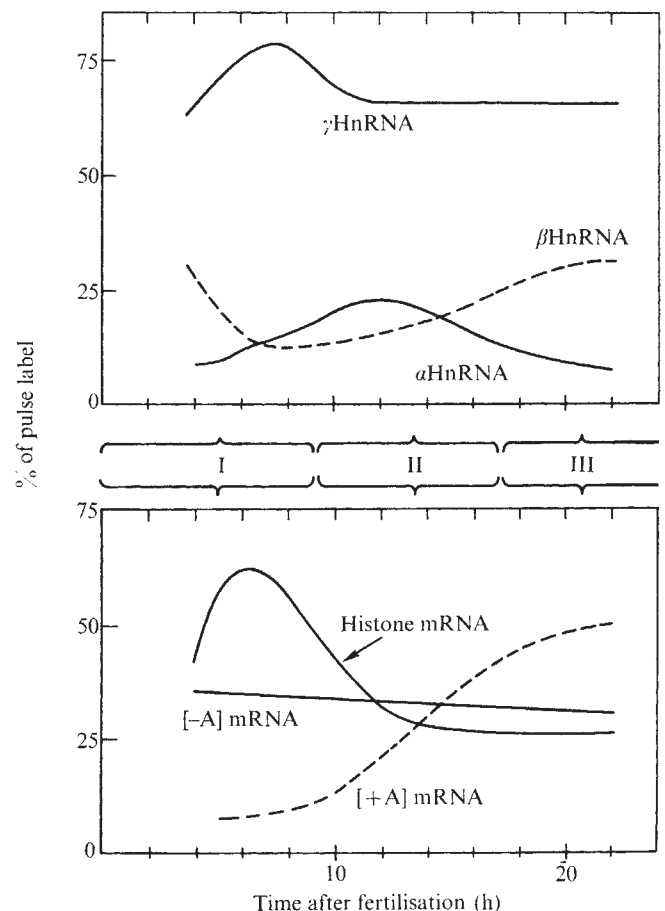
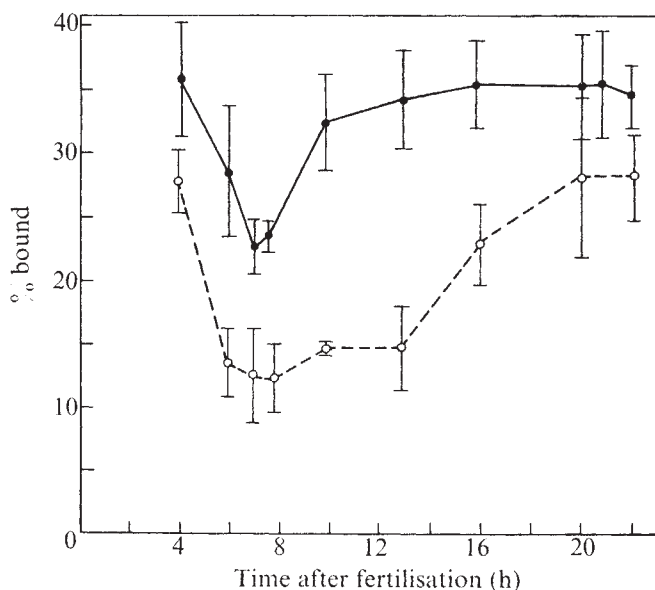


Fig. 3 Changes in the percentage incorporation in HnRNA and mRNA classes during development. The curves in Fig. 2 are used to generate continuous developmental curves of α , β , and γ HnRNA. α HnRNA is measured as the difference between poly(U) filter binding and oligo(dT)-cellulose binding. β HnRNA is that which binds to oligo(dT)-cellulose, and γ HnRNA is that not bound to poly(U). Included also are the curves for relative incorporation of uridine in mRNA (non-4S and non-ribosomal RNA in polyribosomes) for 9S putative histone mRNA, poly(A)-containing mRNA ([+A] mRNA) and mRNA lacking poly(A) ([−A] mRNA), according to Nemer¹¹. Between the developmental curves, three characteristic developmental periods are approximated: I, an early period of rapid cell division of the morula and early blastula; II, a middle period of relative quiescence that includes rotation of the embryo and hatching from its membrane. During this period the embryo is uniquely sensitive to actinomycin treatment, in so far as gastrulation is later inhibited. III, A period of development of the mesenchyme blastula leading into gastrulation.

pattern of HnRNA synthesis. During the first period, from cleavage to early blastula (to approximately 9 h), relative incorporation in γ HnRNA, the most prevalent component, attains a maximum value. HnRNA composition during the early part of this period is uncertain, due to the variable degradation of 4-h morula HnRNA noted in Table 1. Furthermore, the relative contributions of α and β HnRNAs are slightly lower than represented by the method of differential binding (Fig. 2), due to the presence of the δ component (Fig. 1a and b). The relative amounts of α and β HnRNA were therefore quantified from elution profiles, such as those in Fig. 1. They have similar amounts of incorporation at the 7-h early blastula stage and their sum displays a minimum value inversely related to the γ HnRNA maximum. At stages beyond 9–10 h, the definition and quantitation of α and β HnRNA by the method of differential binding is fairly unambiguous. In the second period, from the mid-blastula (10 h) to hatched blastula (16 h), α HnRNA reaches a maximal value and declines; γ HnRNA has a constant value throughout this and the next period;

β HnRNA increases gradually. In the third period, involving development through mesenchyme blastula (22 h), β HnRNA increases sharply and α HnRNA continues to decline.

HnRNA changes and cellular physiology

These results show that different molecular types of HnRNA can be distinguished. Furthermore, relative incorporation in the distinct HnRNA classes, which we have designated α , β and γ , varies as a function of embryonic development. Control experiments indicate that this variation does not reflect preparational artefacts of degradation or contamination. Considering that the 60-min labelling time is at least four times longer than the turnover time for total nuclear RNA¹⁰, these changes in relative incorporation can be regarded as changes in steady-state populations rather than metabolic turnover differences among the classes at different stages. In addition, they may reflect precursor-product relationships among different nuclear and cytoplasmic RNA classes. The nature and timing of developmental shifts in the synthesis of each of these classes may furnish clues about their separate functions.

(1) The relative syntheses of β HnRNA and poly(A)-containing mRNA¹¹ increase concomitantly during development from early blastula to mesenchyme blastula (Fig. 3). These changes might be expected to support changes in protein synthesis that accompany extensive and obvious morphogenetic differentiation, especially the appearance of the three germ layers. The changes in HnRNA may be related to changes in mRNA synthesis, involving selective transcription, nuclear poly(A) polymerase activity¹² and the processing of mRNA precursor molecules.

(2) The relative rate of synthesis of γ HnRNA attains a peak value at the early blastula stage, a time of maximal rate (per embryo) of DNA and histone synthesis¹³, and of the relative synthesis of putative histone mRNA (Fig. 3). This lends credence to the proposition that histone mRNA, which lacks poly(A), is derived from precursors in the poly(A)-deficient γ HnRNA class. Only a small portion of the γ HnRNA, however, can be involved, since γ HnRNA continues to represent the most prevalent HnRNA component even in later stages, when histone and histone mRNA syntheses are much less prominent. The bulk of γ HnRNA seems to be involved in processes other than histone mRNA synthesis.

(3) The relative incorporation in α HnRNA peaks at the mid-blastula stage, the period between the beginning of rotation within the fertilisation membrane and hatching. This seems to be an interval of relative quiescence, following the period of intense cell division (and γ HnRNA synthesis) and preceding

the events leading to gastrulation (including increased β HnRNA synthesis). But it is during this period only that inhibition of transcription results in a block of gastrulation, which occurs much later^{6,7}. Thus, this is a critical period of RNA synthesis for the occurrence of a complex future developmental event. There is no evidence for or against the involvement of any individual RNA class. If, however, we consider the developmental changes in the three classes of HnRNA and the three (not necessarily homologous) classes of mRNA in Fig. 3, we must conclude that only α HnRNA attains a characteristic prominence during this critical period. The circumstantial evidence in this chronology suggests the novel hypothesis concerning the role of transcription in the programming of development: that a special class of HnRNA molecules (α HnRNA) functions to predetermine the outcome of later developmental events (gastrulation).

The involvement of α HnRNA could entail the recognition of distinct chemical properties¹, consistent with the operation of developmental signals. It remains to be determined whether or not translatable nucleotide sequences are present in α HnRNA. If this were so, then the critical function of α HnRNA might be to supply templates for immediate or future translation. Alternatively, the regulatory function of such an HnRNA class might not depend on its giving rise to mRNA, but on its intrinsic properties. Theoretically, HnRNA molecules or their derivatives might activate and coordinate gene activity¹⁴, influence mRNA translation¹⁵, or interact with chromatin and influence chromosomal structure^{16,17}.

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- ¹ Dubroff, L. M., and Nemer, M., *J. molec. Biol.*, **95**, 455-476 (1975).
- ² Gross, P. R., and Cousineau, G. H., *Expl Cell Res.*, **33**, 368-395 (1964).
- ³ Nemer, M., and Infante, A., *Science*, **150**, 217-221 (1965).
- ⁴ Kedes, L. H., and Gross, P. R., *J. molec. Biol.*, **42**, 559-575 (1969).
- ⁵ Terman, S., *Proc. natn. Acad. Sci. U.S.A.*, **65**, 985-992 (1970).
- ⁶ Barros, C., Hand, G., Jr, and Monroy, A., *Expl Cell Res.*, **43**, 167-182 (1966).
- ⁷ Guidice, G., Mutolo, V., and Donatut, G., *Wilhelm Roux Archiv.*, **161**, 118-128 (1968).
- ⁸ Johnson, L., Williams, J., Abelson, H., Green, H., and Penman, S., *Cell*, **4**, 69-75 (1975).
- ⁹ Dubroff, L. M., dissertation, Univ. Pennsylvania (1975).
- ¹⁰ Brandhorst, B. P., and Humphreys, T., *J. Cell Biol.*, **53**, 474-482 (1972).
- ¹¹ Nemer, M., *Cell*, **6**, 557-568 (1975).
- ¹² Hyatt, E. A., *Biochim. biophys. Acta*, **142**, 246-253 (1967).
- ¹³ Moav, B., and Nemer, M., *Biochemistry*, **10**, 881-888 (1971).
- ¹⁴ Britten, R., and Davidson, E., *Science*, **165**, 349-357 (1969).
- ¹⁵ Bester, A. J., Kennedy, D. S., and Heywood, S. M., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1523-1527 (1975).
- ¹⁶ Crick, F., *Nature*, **234**, 25-27 (1971).
- ¹⁷ Pettijohn, D., and Hecht, R., *Cold Spring Harb. Symp. quant. Biol.*, **38**, 31-41 (1973).
- ¹⁸ Scarano, E., and Maggio, R., *Expl Cell Res.*, **18**, 333-346 (1959).

letters to nature

Test of pulsar acceleration mechanisms

AN elegant mechanism to explain the high velocities of pulsars has been proposed by Harrison and Tademaru^{1,2}. Based on the observations that the interpulses seen in some pulsars are located asymmetrically between the main pulses, they assume that pulsar magnetic fields arise in general from off-centred dipoles. The asymmetric radiation reaction produced by such an inclined off-set rotating dipole accelerates the pulsars in a direction parallel to their spin axes and gives the observed velocities. Any verification of the above hypothesis hinges on the possibility of determining, and comparing, the angle projected by the spin axis on the sky S with the observed

direction of motion of the pulsar V . According to Harrison and Tademaru's model^{1,2}, these directions would coincide ($V = S$). We wish to point out here that a measure of the spin axis projected direction is given by the intrinsic angle of polarisation of the radio emission at the centre of the integrated pulse profile, and that these polarisation data do not support the hypothesis in most cases.

It may be remarked here that in those pulsars from which asymmetrically located inter-pulses are seen, this mechanism will be weak, if operative at all, since the magnetic dipole is almost at right angles to the rotational axis. Furthermore, asymmetrically located inter-pulses cannot result from merely

Table 1 Data for $\phi_1 - V$ for pulsars (See text for details)

Pulsar	SNR	V		ref.	ϕ_1	ref.	$\phi_1 - V$
0329+54	CTB13 ??	293 $\pm$ 15	Sc	7	80 $\pm$ 10	10	33 $\pm$ 18
		126 $\pm$ 8	PM	7			46 $\pm$ 13*
0525+21	S147?	203 $\pm$ 7	D		40 $\pm$ 10	10	17 $\pm$ 12
0531+21	Crab	294 $\pm$ 8	PM	5	145 $\pm$ 20	10	31 $\pm$ 22
0611+22	IC443	274 $\pm$ 10	D		150 $\pm$ 70	16	56 $\pm$ 71
0823+26		158 $\pm$ 3	PM	7	73 $\pm$ 6	17,16	85 $\pm$ 7
0833-45	Vela X	234 $\pm$ 15	D	†	47 $\pm$ 10	18	7 $\pm$ 18
0834+06		-19 $\pm$ 10	PM	7	105 $\pm$ 80	10	86 $\pm$ 81
1133+16		0 $\pm$ 15	PM	6	93 $\pm$ 10	14,10	87 $\pm$ 18
		-10 $\pm$ 4	PM	7			77 $\pm$ 11
1237+25		75 $\pm$ 4	PM	7	0 $\pm$ 15	10	75 $\pm$ 15
1749-28	W28?	200 $\pm$ 5	D		9 $\pm$ 8	14,16	11 $\pm$ 10
1929+10	CTB72?	22 $\pm$ 8	D		64 $\pm$ 6	14,10	42 $\pm$ 10
2016+28		9 $\pm$ 13	PM	7	100 $\pm$ 50	10	89 $\pm$ 51
2021+51	HB21?	287 $\pm$ 5	D		50 $\pm$ 7	15,16	57 $\pm$ 9

*Adopted value.

†Using maps at 408 MHz and 635 MHz.

offsetting a dipole field; the direction of emergence of the field lines from the surface must be modified such that these directions for the two poles are not at 180°.

Competing acceleration mechanisms predict different relations between V and S . For example there is evidence that in visual binaries the spin axes of the individual stars are preferentially aligned perpendicular to the orbital plane³. The sling shot acceleration mechanism for runaway stars proposed by Blaauw would then produce motion perpendicular to the spin axes of the stars ($V = S + 90^\circ$), if a similar alignment takes place in close binary systems producing pulsars. On the asymmetric supernova explosion hypothesis discussed by Shklovsky and Michel⁴, it is not clear if a preferred direction of ejection relative to the spin axes would be expected.

The angle V can be found from proper motion measurements⁵⁻⁷, from the angular displacement of pulsars from their associated supernova remnants, or from interstellar scintillation observations⁷. We have collected the available measures of V and ϕ_1 , the intrinsic angle, and on this basis we will assess here the relative importance of the various proposed acceleration mechanisms.

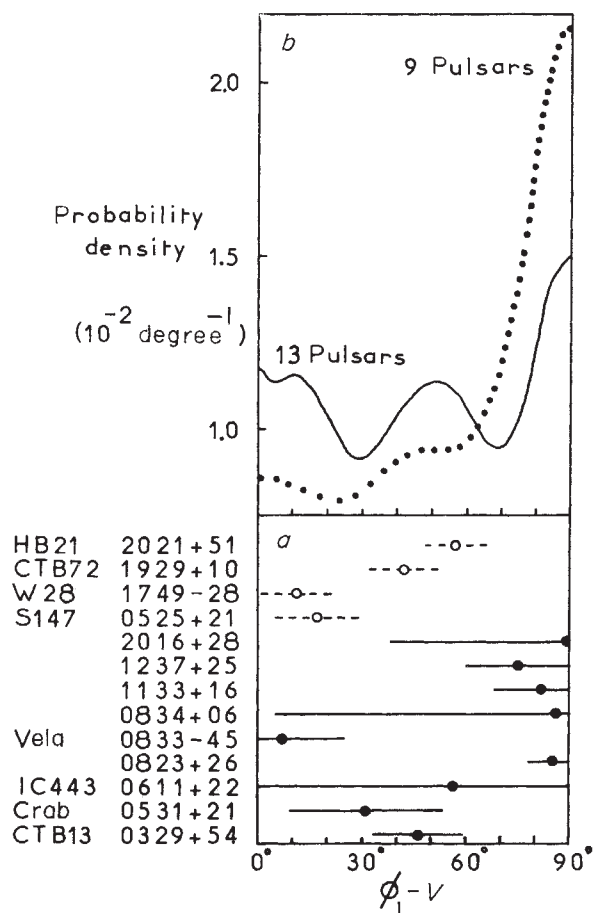
We make two general assumptions: firstly, as in most theories of pulsar radio emission, we attribute the systematic sweep of the plane of the average polarisation to the changing aspect of the projected magnetic field in the radiating region as the pulsar rotates, and assume that there is a constant relation between the projected field direction and the plane of polarisation. Secondly, we assume that at pulse maximum, there will be a constant difference between the projected magnetic field direction (and hence the plane of polarisation) and the projected direction of the spin axis, independent of other parameters such as velocity of rotation or inclination of the spin axis to the plane of the sky. In such cases $\phi_1 = S + k$ can be used as a measure of the projected spin axis.

There is evidence in the case of the Crab Nebula that the constant k is zero. If the magnetic field in the nebula as a whole is wrapped toroidally about the pulsar spin axis⁸, then measurements⁹ of the optical polarisation of the nebula imply a spin axis at position angle 140°. This is in close agreement with the intrinsic position angle¹⁰ of the pulsar radio emission (145 $\pm$ 20°). It may be noted that the value $k = 0$ is just that predicted by those polar cap models¹¹ in which the radio emission comes from (coherent) curvature radiation. Thus, in summary, for many pulsar models $\phi_1 = S + k$, and a preferred value of $\phi_1 - V$ would imply a directed ejection of pulsars from supernovae. If, as seems likely, $k = 0$, then a preferred value of $\phi_1 - V = 0^\circ$ is expected from Harrison and Tademaru's hypothesis, and $\phi_1 - V = 90^\circ$ for the sling shot or runaway star mechanism.

Table 1 gives the available values of $\phi_1 - V$, expressed

1180°. The supernova that has been associated with each pulsar is indicated in column 2, with ? to indicate doubtful cases. The type of observation leading to the adopted value of V (column 3) is shown in column 4. Sc denotes an interstellar scintillation measurement, PM a measured proper motion

Fig. 1 *a*, Plot of the difference ($\phi_1 - V$) between the intrinsic position angle of polarisation ϕ_1 at the centre of the pulse profile and the direction of proper motion V . Values dependent on doubtful associations are dotted. *b*, The differences $\phi_1 - V$ of Fig. 1*a* plotted as a probability distribution, taking into account the different weights of the various measurements as given in column 8 of Table 1. —, All data;, with uncertain supernova remnant associations excluded.



(either by pulse-timing or interferometry), and D indicates a measured displacement of the pulsar from the centre of the associated supernova remnant. Pulsar positions were taken from the compilation of Manchester and Taylor¹², and the supernova remnant positions were obtained from published radio maps. The source of the values of ϕ_1 (column 6) is indicated in column 7. In some cases, lower errors in extrapolating to zero wavelength have been obtained by taking high frequency observations¹³⁻¹⁵ and correcting for Faraday rotation by using published Rotation Measures derived independently from low-frequency observations^{10,16}. For these pulsars two references are given in column 7. For PSR2021+51 a position angle of polarisation of $38 \pm 5^\circ$ at 1,665 MHz has been taken from ref. 15 and corrected using a Rotation Measure¹⁶ of -6.5 m^{-2} .

The values of $\phi_1 - V$ (in the range 0° – 90°) from Table 1 are displayed in Fig. 1a and are shown as a probability distribution in Fig. 1b in an attempt to allow for the different weights of the various measurements. There is a preponderance of values of $\phi_1 - V$ close to 90° in the full curve containing all the data. The peak in probability density at $\phi_1 - V = 0$ in Fig. 1b comes largely from data for pulsar-supernova associations which are not very well established, S147, W28, HB21 and OTB72. If these data are not included (dotted line), then the preferred direction $\phi_1 - V = 90^\circ$ becomes much more pronounced. If we now apply the condition that the polarisation and the spin axis are related as predicted by the curvature radiation polar cap models, and supported by observations of the Crab Nebula and the pulsar PSR0531+21, this correlation indicates that the acceleration mechanism proposed by Harrison and Tademaru is clearly not dominant.

The distribution in Fig. 1b seems to favour a 'sling shot' or similar mechanism or a 'spin-aligned' ejection of pulsars from supernovae. The scatter of values about 90° can be attributed to observational errors, the residual influence of the Harrison and Tademaru mechanism, or more likely to random effects of which at least two can be envisaged. Firstly, any significant proper motion of the parent star before the supernova explosion will make a contribution to the motion of the pulsar. Secondly, an intrinsic scatter of the spin angular momentum of the parent star about the normal to its orbital plane can be expected on the sling-shot hypothesis.

Although the dotted line distribution of Fig. 1b is clearly peaked at 90° , we note that for the two pulsars whose association with supernovae is least questioned, (the Crab and Vela), $\phi_1 - V$ is very close to zero and in fact responsible for the tail of the distribution. This raises the possibility that there might be a difference between pulsars associated with supernovae and those not. Support for the possibility that not all pulsars are created in supernova explosions is provided by the case of the binary pulsar PSR1913+16 which has no observable associated remnant, although it is the second fastest pulsar known¹⁷. Perhaps the asymmetric radiation reaction mechanism operates only on pulsars created in supernovae, because the rotational speeds attained initially might be very much higher than those for pulsars created through accretion in binaries, for example.

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D. MORRIS

Max-Planck Institute für Radioastronomie
53 Bonn 1,
Auf dem Hügel 69, West Germany

V. RADHAKRISHNAN
C. SHUKRE

Raman Research Institute,
Bangalore—560 006,
India

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- ¹ Harrison, E. R., and Tademaru, E. P., *Nature*, **254**, 276 (1975).
- ² Harrison, E. R., and Tademaru, E. P., *Astrophys. J.*, **201**, 447 (1975).
- ³ Weiss, E. R., *Astrophys. J.*, **190**, 331 (1974).
- ⁴ Tsarevsky, G. S., *Astrophys. Lett.*, **10**, 71 (1972).
- ⁵ Trimble, V., *IAU Symp.* No. 46, 12 (1970).

- ⁶ Manchester, R. N., Taylor, J. H., and Van, Y. Y., *Astrophys. J., Lett.*, **189**, L119 (1974).
- ⁷ Anderson, B., Lyne, A. G., and Peckham, R. J., *Nature*, **258**, 215 (1975).
- ⁸ Rees, M. J., and Gunn, J. E., *Mon. Not. R. astr. Soc.*, **167**, 1 (1974).
- ⁹ Disney, M. K., *Astrophys. Lett.*, **9**, 9 (1971).
- ¹⁰ Manchester, R. N., *Astrophys. J.*, **172**, 43 (1972).
- ¹¹ Komesaroff, M. M., *Nature*, **225**, 612 (1970).
- ¹² Manchester, R. N., and Taylor, J. H., *Astrophys. Lett.*, **10**, 67 (1972).
- ¹³ Komesaroff, M. M., Morris, D., and Cooke, D. J., *Astrophys. Lett.*, **5**, 37 (1970).
- ¹⁴ Morris, D., Schwarz, U., and Cooke, D. J., *Astrophys. Lett.*, **5**, 181 (1970).
- ¹⁵ Manchester, R. N., *Astrophys. J. Suppl.*, **23**, No. 199 (1971).
- ¹⁶ Manchester, R. N., *Astrophys. J.*, **188**, 637 (1974).
- ¹⁷ Van den Bergh, S., *Astrophys. Lett.*, **16**, 75 (1975).
- ¹⁸ Radhakrishnan, V., and Cooke, D. J., *Astrophys. Lett.*, **3**, 225 (1969).

Angular momentum diffusion and the initiation of cyclones

It is possible to explain^{1,2} the intensification of a cyclone vortex once the stream lines in a weak depression close around a core of higher vorticity, but it is less clear how the process starts. Scorer³ proposed that convection might diffuse angular momentum within a field of initially uniform rotation to localised regions, thereby concentrating cyclonic vorticity. Experiments failed to demonstrate the effect⁴⁻⁷, and although strong vortices could be produced through the breaking of inertial waves⁸, the effects were open to misinterpretation. We present here new experimental results showing that first, angular momentum or relative vorticity can be diffused at a rate comparable with the diffusion of relative velocity (turbulent 'viscosity'). This distinctive property seems to be intrinsic to weak turbulence in rotation, and can be parameterised very simply. Second, such diffusion occurs only when the turbulence is sufficiently weak to be constrained by rotation to an essentially two-dimensional structure. This might explain the failure of earlier experiments. Our results could have many applications to geophysics, including tropical cyclogenesis.

The experiments were conducted in a transparent, plane-ended cylinder of uniform height h (22.8 cm) and radius a (14.8 cm) mounted axially on a turntable (with rotation rates Ω between 3 and 11 rad s⁻¹), completely filled with a homogeneous fluid (water, or a glycerine water solution). The bottom was regularly perforated by 199 holes of 2.21 mm bore, at 2 cm spacing. Each hole connected through tubing to one of two manifolds, 49 holes at 4-cm regular spacing going to one and the remainder to the other (Fig. 1a). Fluid (with a few polystyrene beads suspended in it for visibility) was pumped between the manifolds to induce a radially uniform, regular, small scale mixing pattern without mean advection in the radial plane. The convective geometry was varied by closing some jets or by pump reversal. Figure 1b shows array geometries. In some experiments using array III, a false top was put in the container to vary h .

Horizontal velocities in the central third of the cylinder were measured photographically. Without rotation, the jets caused the paths shown in Fig. 2a. Figure 2b shows the effect of rotation with the same jet conditions. Particle concentrations reveal intense vortices, always cyclonic, drifting slowly within the rotating frame, apparently through mutual interaction or by weak relative rotation of the fluid body (usually $<0.01 \Omega$), independently of their position relative to the jets. The structure was usually well established within 30 rotation periods after pumping started. Once formed, vortices usually kept their identity. They merged occasionally, but others would form to keep the population constant. The vortices aligned themselves parallel to the rotation axis and extended from top to bottom.

Since particle paths were roughly circular and coaxial near the vortices, a mean relative circumferential velocity v could be measured as a function of local radius r from the origin of the vortex centre. This was expressible as an absolute azimuthal velocity $V = \Omega r + v$ in an irrotational frame.

Randomly chosen vortices were measured for a variety of conditions of Ω , h , pumped volume flux M , number of jets N , and kinematic viscosity ν . The profile at V as a function of r plotted logarithmically, had a similar shape for all results

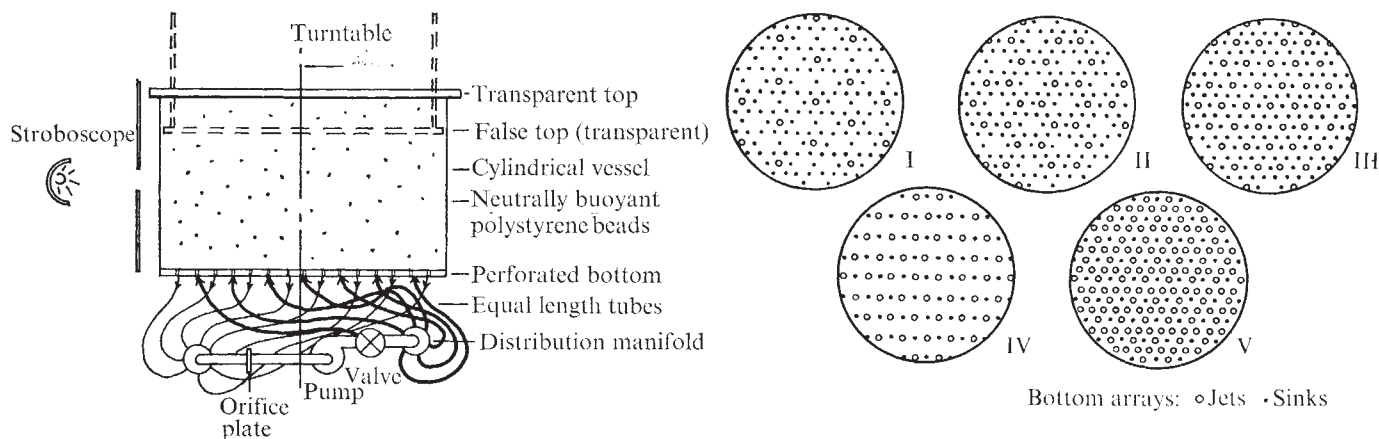


Fig. 1 Experimental arrangement and pumping arrays.

and thus could be aligned, within experimental accuracy, to a 'similar' profile (Fig. 3a).

$$V/Q = f(r\Omega/Q) \quad (1)$$

when scaled by a suitable parameter Q having the dimensions of velocity. Q was found to depend linearly on $w\rho/\delta$, where w is the vertical convective velocity scale ($= M/\pi a^2$), ρ is the r.m.s. jet spacing ($= aN^{-1/2}$) and δ is the scale of the viscous boundary layer on the end walls, defined here as $(\nu/\Omega)^{1/2} + w/2\Omega$. As a limit to the magnitude of Q , coherent vortices seldom formed when

$$w_j/\Omega h > 1.15 \pm 0.05 \quad (2)$$

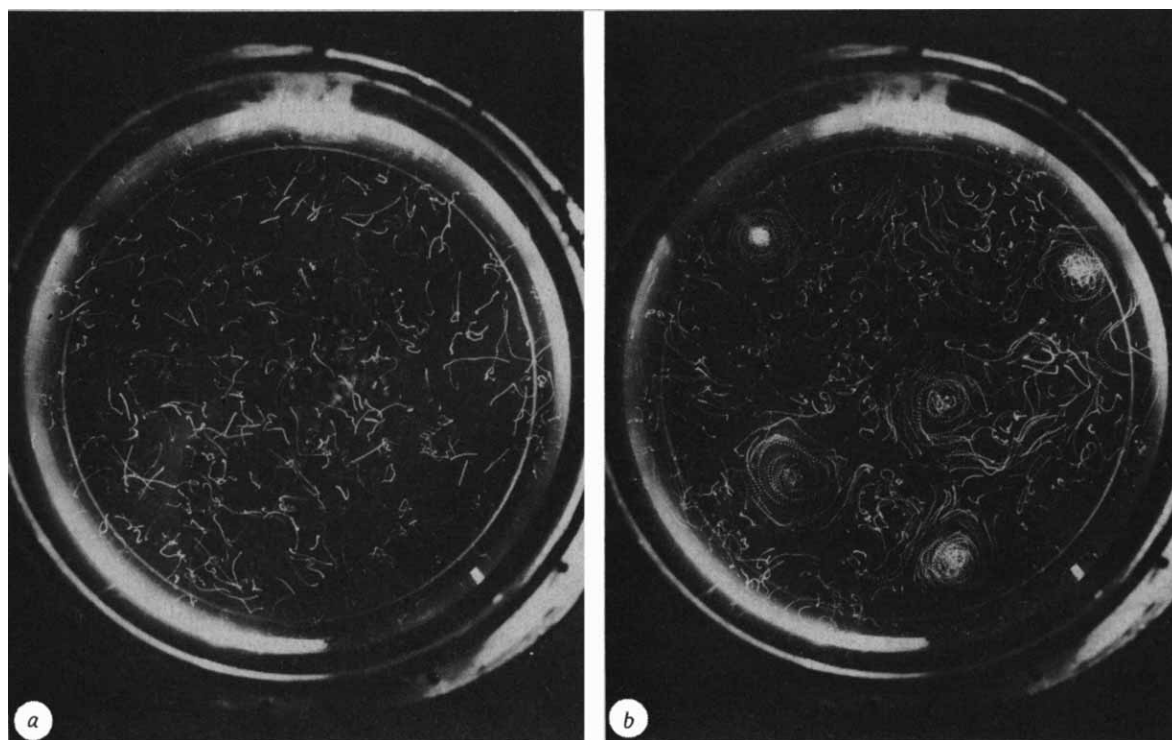
where $w_j = w\rho^2/a_j^2$ defines a mean maximum vertical velocity at jet exit and a_j is the jet radius.

Every feature of these results can be explained as follows:

To conserve angular momentum in a steady axisymmetric system the torque coming from the turbulent stress must balance the momentum change caused by the radial divergence, i.e., $d(Tr^2)/dr = \gamma U r d(Vr)/dr$ where T is the mean tangential stress and γ is density; the radial mean velocity U is induced by end-wall boundary layers and has magnitude $\nu\delta/h$.

At its simplest, externally imposed turbulence is characterised by a single horizontal velocity scale q . T may depend both on q and the mean flow quantities, and by dimensional considerations, 'universal' similarity profiles as in equation (1) can generally occur only when Q is proportional to qh/δ . If, through rotational constraint, the turbulence approaches two-dimensionality, horizontal and vertical velocity scales become simply related and $q \propto w\rho/\delta$, consistent with equation (2), so $Q = c w\rho/\delta$ (c is some constant) precisely as observed.

Fig. 2 Particle movements, ~ 1 s exposure in 50-Hz stroboscopic light by a camera mounted axially on the turntable, with array III; pumping rate for both figures is the same, corresponding to $w = 0.3 \text{ cm s}^{-1}$. *a*, No rotation. Particle movements are random and short in scale. *b*, Rotation 8.24 rad s^{-1} , after pattern development. Intense cyclonic vortices, dissociated from the bottom jets, are sustained.



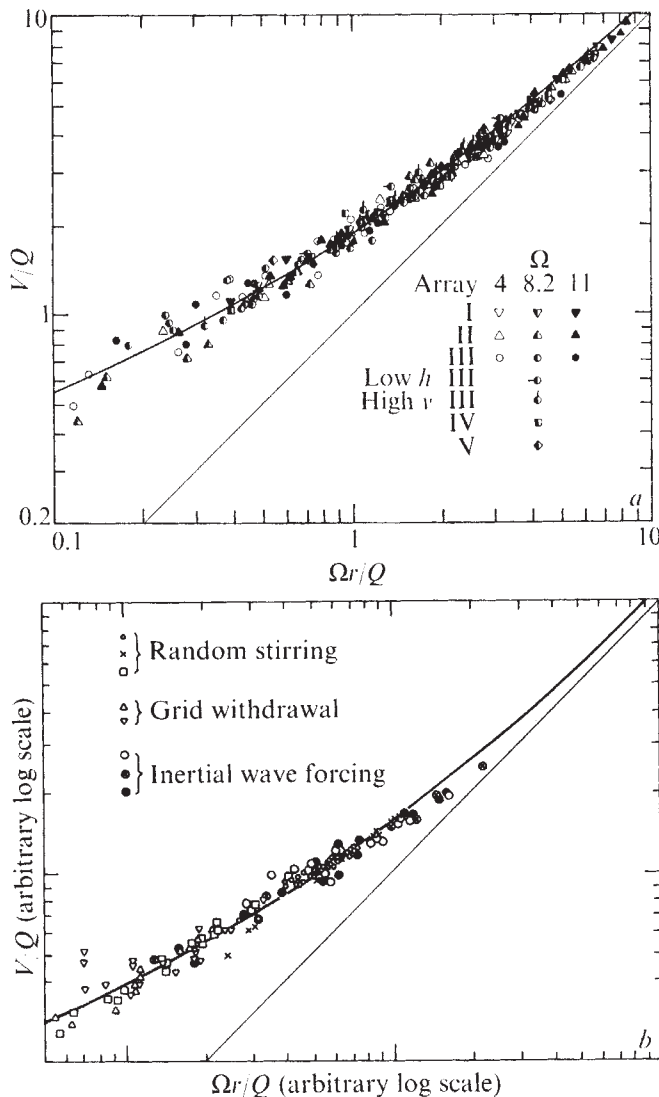


Fig. 3 Absolute azimuthal velocity V as a function of radius r from vortex core, for experimental conditions listed (logarithmic coordinates). V and r are scaled for each data set by velocity Q . Solid rotation Ω is delineated by a faint solid line. The bold line is the theoretical 'universal' absolute velocity profile arising with a constant ratio (2/3) between angular momentum diffusivity and turbulent 'viscosity'. *a*, Randomly chosen vortices with the experimental arrangement described; *b*, with alternative mixing methods: (1) after random excitation, $\Omega = 1.4 \text{ rad s}^{-1}$; (2) after sudden axial withdrawal of a grid comprising 1-cm-wide battens in a $4 \times 4 \text{ cm}$ square plane array (performed by M. Riefman); (3) Continuous resonant forcing of inertial waves following the method of McEwan⁸ (performed by W. McKimm) $\Omega = 8.2 \text{ rad s}^{-1}$.

The essential influence of rotation is reflected in the law defining T , the mean tangential stress. If angular momentum were conserved during radial mixing the quantity, T would vary linearly with $r^{-1}d(Vr)/dr$, but in isotropic turbulence behaving like laminar viscosity, T would be proportional to $rd(Vr^{-1})/dr$. Between these extremes universal profiles arise only when the two terms are in constant proportion $\xi : 1$. Then equation (1), with T so specified, can be solved numerically. The curve of best fit, $\xi = 2/3$, $C = 0.82$ is shown in Fig. 3*a*. Figure 3*b* shows the fit to vortices produced after cessation of mechanical stirring, or by inertial wavebreaking as used before⁸. Agreement is uniformly good. This explanation implies that the eddies of rotationally dominated turbulence are constrained to a specific anisotropic form. This explains how the diffusion properties can be characterised by a single velocity scale. Note that positive potential vorticity⁹ diffusion could not yield our profile.

The significance of the foregoing in tropical cyclogenesis is gauged by considering a disturbance in which the net atmospheric rotation is $2.5 \times 10^{-5} \text{ s}^{-1}$, $h = 12 \text{ km}$, w is 1 cm s^{-1} and 3 cm s^{-1} between clouds at cloud base and in the deep convection zone respectively. Cloud spacing $2\rho = 20 \text{ km}$ and δ is $\sim 200 \text{ m}$.

The depression would then be intensified significantly by 0.5 mbar over a 570-km radius, and the vorticity increased to $1.4 \times 10^{-4} \text{ s}^{-1}$ at a 10-km radius in a one-week time scale, independently of any thermodynamic advective effects. The condition in equation (2) is exceeded in active cloud columns where $w_j/\Omega h$ is ~ 10 , but not in the intercloud volumes. Considering the ease with which the controlling variables can be quantified, further observation and simulation studies seem worthwhile.

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A. D. McEWAN

CSIRO Division of Atmospheric Physics,
Aspendale, Victoria,
Australia

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- 1 Charney, J. E., and Eliassen, A., *J. Atmos. Sci.*, **21**, 68–75 (1964).
- 2 Carrier, G. F., *J. Fluid Mech.*, **11**, 145–158 (1971).
- 3 Scorer, R. S., *Sci. J.*, **2**, 46–52 (1966).
- 4 Gough, D. O., and Linden-Bell, D., *J. Fluid Mech.*, **32**, 437–447 (1968).
- 5 Bretherton, F. P., and Turner, J. S., *J. Fluid Mech.*, **32**, 449–464 (1968).
- 6 Strittmatter, P. A., Illingworth, E., and Freeman, K. C., *J. Fluid Mech.*, **40**, 737–751 (1970).
- 7 Ibbetson, A., and Tritton, D. J., *J. Fluid Mech.*, **68**, 639–673 (1975).
- 8 McEwan, A. D., *Geophys. Fluid Dynam.*, **5**, 283–311 (1973).
- 9 Welander, P., *Geophys. Fluid Dynam.*, **5**, 173–189 (1973).

Rock volatility and aerosol composition

ON the basis of recent measurements, I propose here that part of the Earth's aerosol burden comes from the volatilisation (sublimation) of the components of surface rocks, in addition to the well known man-made contribution. Calculations on a simple kinetic model indicates that vapour pressures of $\sim 10^{-11} \text{ atm}$ could support the observed atmospheric concentration of copper.

Analyses of the inorganic constituents contained in aerosols and rains show a remarkable similarity in abundance patterns (Table 1). In general, the sequence of decreasing concentrations for a group of heavy metals is: Pb, Zn, Cu, Mn and Ni. The measurements cited were made in both hemispheres and on several continents, in urban and rural areas and in all seasons of the year. It is thus difficult to relate these findings to pollution sources, particularly for the heavy metals in the South Pole atmosphere¹. The above sequence does not resemble that found in crustal rocks or soils (Table 1), where, for example, the concentration of manganese is an order of magnitude greater than that of the other four metals.

Two lines of evidence support the hypothesis that the fractionation responsible for such distributions involves a selective volatilisation. First, emission spectrographers have long been aware of the selective volatilisation of the elements and their compounds². This depends on the matrix in which the elements are bound. For sulphides, oxides, sulphates, carbonates, silicates, and phosphates, the distillation rankings are similar: Pb, Zn, Cu, Mn and Ni, listed in order of decreasing volatility. This applies, however, to extrapolation from high temperatures: vapour pressures for lower temperatures have not yet been measured for this suite of metals and their compounds (there are isolated measurements of only some of the more volatile species). Also, these metals exist in nature in forms other than those used for emission spectroscopic studies. Nonetheless, the matching of the two sequences seems significant.

Kinetic theory shows that even their low vapour pressures are sufficient to account for steady state fluxes of these species

Table 1 Trace metal concentrations in aerosols, rains and crustal rocks*

	Pb	Zn	Cu	Mn	Ni
Mean concentrations in South Pole atmosphere ¹ (10^{-12} g m ⁻³)	630	30	36	10	
Average concentrations in aerosols collected in San Francisco Bay area ¹² (10^{-9} g m ⁻³)		136	50	17	
Average concentrations in US precipitation ¹³ (parts per 10 ⁹)	34	107	21	12	4
Average concentrations in Tucson air ¹⁴ (10^{-6} g m ⁻³)	0.47	0.15	0.41	0.013	0.021
Average concentrations in rain at Wraymires, UK ¹⁵ (10^{-6} g l ⁻¹)	39	85	23	8	6
Average concentrations in air at Wraymires ¹⁵ (10^{-9} g kg ⁻¹)	87	80	26	11	6
Crustal abundance ¹⁶ (p.p.m.)	10-15	32-58	37-72	670-1,000	37-72
Soils ¹⁷ (%)	1×10^{-3}	5×10^{-3}	2×10^{-3}	6.7×10^{-3}	4×10^{-3}

*Concentrations are taken directly from references and have not been corrected to a simple set of units in as much as their order is of significance.

to the atmosphere³. The rate of loss of a substance, W , from a surface is given by

$$W = p (M/2\pi RT)^{1/2}$$

where p is the vapour pressure of the substance with molecular weight M , and T and R are the absolute temperature and gas constant respectively. Multiplying W by σ , the land surface area in cm² (3.14×10^{18}) and by f , the fraction of the substance in crustal material gives a continental flux. This may be compared with a flux value calculated to give an observed atmospheric burden A in the troposphere, where its tropospheric residence time τ is determined by the removal of the substance with rain. The flux Φ is $\Phi = A/\tau$

then

$$A/\tau = p(M/2\pi RT)^{1/2} \sigma f$$

For a substance whose concentration in the atmosphere is C (g cm⁻³) and the volume of the atmosphere is v , $A = Cv$, but v/σ is the height of the atmosphere h (the average tropospheric height is taken as 13 km). Thus, the equation reduces to

$$p = (h/\tau)[2\pi(RT/M)]^{1/2}(C/f)$$

If τ is taken as ten days, we get

$$p = 3.4 \times 10^{-2}(T/M)^{1/2}(C/f)$$

Computed values of p may be obtained from the data in Table 1. For Cu, an atmospheric concentration of 50×10^{-15} g cm⁻³, and a crustal abundance of 50 p.p.m. yields vapour pressures for its species of 6.6×10^{-11} atm at 300 K and 12×10^{-11} atm at 1,000 K. We note that DDT has been dispersed around the Earth primarily in the vapour phase⁴, and its vapour pressure is $\sim 2 \times 10^{-10}$ atm (ref. 5) at 300 K. Its mean concentrations in the air over the Sargasso Sea⁴ were $\sim 0.42 \times 10^{-16}$ g cm⁻³.

The role of sublimation in dispersing chemical species around the Earth in the major sedimentary cycle may be significant for such elements as mercury, selenium and arsenic. Weiss *et al.*⁶, from rain and atmospheric analyses, suggest that the amount of mercury entering the atmosphere from the degassing of the lithosphere is $\sim 0.25 \times 10^{11}$ – 1.5×10^{11} g yr⁻¹. The flux of mercury from the continents to the oceans in rivers appears to be much less than that of the continents to the atmosphere. The former flow is estimated to be $\leq 3.8 \times 10^9$ g yr⁻¹. Weiss *et al.*⁶ also surveyed man-made mercury pollution. Most probably comes from sulphide mining, which could give a flux of 2×10^9 g yr⁻¹. Thus natural degassing may be a primary path of mercury from the continents to the atmosphere and thence to the oceans.

Atmosphere particulates, collected from the northern, equatorial and southern Atlantic Ocean, southern and eastern China Sea, and various coastal localities, have concentrations (on a gram per gram basis) of Pb, Zn and Sn which are one order of magnitude higher than those in average crustal materials⁷, which may be attributed to an anthropogenic

source. These three elements could, however, be introduced also by natural degassing, as many of their compounds are volatile.

There is other evidence to support the volatility hypothesis. A large number of Cu and Pb minerals are associated with fumarolic incrustations compared with the number of Mn and Ni minerals⁸, and high values of Cu and Pb were, in general, found in atmospheric emissions from Hawaiian volcanoes⁹. Also, anomalously high concentrations of Cu, Pb and Zn were observed in rain which fell in the vicinity of sulphide ore deposits in the Maritime Province¹⁰. The rain presumably collected these metals from the atmosphere. I note also that rain can introduce halide ions to rock surfaces. The possible formation and subsequent volatilisation of heavy metal halides can enhance rock volatility in as much as such species have higher vapour pressures than their oxides, carbonates and silicates. The relative volatilities of substances have been used to explain the compositions of lunar soils¹¹.

It therefore would be valuable to determine experimentally the vapour pressures of heavy metals from a suite of common rock types. Such results might lead to more sophisticated field studies to ascertain the role of rock volatility in the major sedimentary cycle and the influence of such processes on the composition of surface seawaters. The upper levels of the mixed layer may receive spikes of volatile chemical species from the continents through rain, whose effect on biological processes could be significant.

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EDWARD D. GOLDBERG

*Scripps Institution of Oceanography,
La Jolla, California 92037*

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- Zoller, W. H., Gladney, E. S., and Duce, R. A., *Science*, **183**, 198–200 (1974).
- Ahrens, L. M., and Taylor, S. R., *Spectrochemical Analysis*, 454 (Addison-Wesley, Reading, Massachusetts, 1961).
- Lloyd-Jones, C. P., *Nature*, **224**, 65–66 (1971).
- Bidleman, T. F., and Olney, C. E., *Science*, **183**, 516–518 (1974).
- Nisbet, I. C. T., and Sarofim, A. F., *Environ. Health Perspectives*, **1**, 21–38 (1972).
- Weiss, H. V., Koide, M., and Goldberg, E. D., *Science*, **174**, 692–694 (1971).
- Chester, R., and Stoner, J. H., *Mar. Chem.*, **2**, 157–188 (1974).
- Stoiber, R. E., and Rose, W. I., Jr., *Geochim. cosmochim. Acta*, **38**, 495–516 (1974).
- Cadle, R. D., Wartburg, A. F., Pollock, W. H., Gandrud, B. W., and Shedlovsky, J. P., *Chemosphere*, **6**, 231–234 (1973).
- Kolotov, B. A., Kiseleva, Ye. A., and Rubeykin, V. Z., *Geochem. Int.*, **2**, 675–677 (1965).
- Chou, C. L., Baedeker, P. A., and Wasson, J. T., *Proc. Fourth Lunar Sci. Conf., Geochim. cosmochim. Acta Suppl.*, **4**, 1523–1533 (1973).
- John, W., Kaifer, R., Rahn, K., and Wesolowski, J. J., *Atmos. Environ.*, **7**, 107–118 (1973).
- Lazrus, A. L., Lorange, E., and Lodge, J. P., Jr., *Environ. Sci. Technol.*, **4**, 55–58 (1970).
- Ranweiler, L. E., and Moyers, J. L., *Environ. Sci. Technol.*, **8**, 152–156 (1974).
- Peirson, D. H., Cawse, P. A., Salmon, L., and Cambray, R. S., *Nature*, **241**, 252–256 (1973).
- Turekian, K. K., and Wedepohl, K. H., *Bull. geol. Soc. Am.*, **72**, 175 (1961).
- Vinogradov, A. P., *The geochemistry of rare and dispersed chemical elements in soils*, 209 (Consultants Bureau Inc., New York, 1959).

Changes in Canadian national visibility

MAN-MADE pollution can have important effects on public health and the climate^{1–3}. The latter, particularly, is a long term effect, and necessitates long term study over wide areas. Here we report the measurement of one relevant

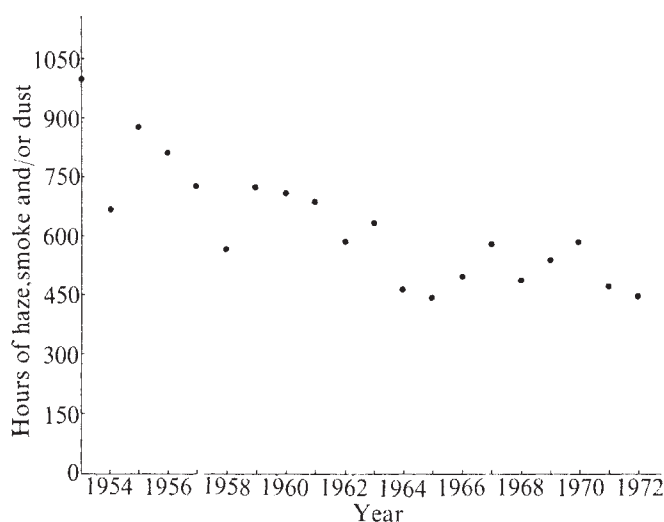


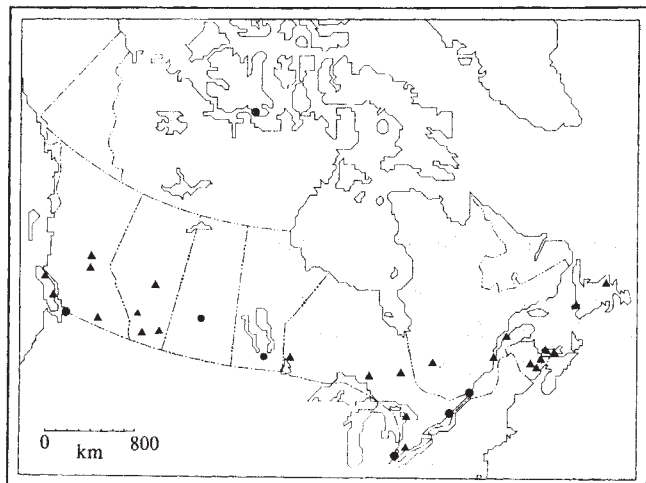
Fig. 1 Weighted average hours of haze, smoke and/or dust per year for major Canadian cities. The data were weighted by the metropolitan populations.

parameter, visibility, at Canadian airports. There are more than 70 usable sets of data. We have found that from 1953 to 1963 there was a general increase in visibility, but, since that date the visibility has remained roughly constant. We attribute both effects to pollution.

Previous work on time trends in visibility and related variables (such as atmospheric turbidity) have generally been limited to a small number of stations and for a short period of time (ref. 4 and other references available on request). Because the data were taken at many different times and places, it is difficult to draw statistically sound judgments on large scale trends. Holzworth (unpublished) has been one of the few to evaluate this quantity on a national basis (for the USA), and determined that visibilities did not deteriorate significantly over a period of ~ 15–20 yr.

Visibility may be affected by a number of pollutants, or change naturally. For example, decreases in visibility have been associated with the presence of ammonia, sulphates, and nitrates^{5,7}, as well as smoke and other particulates, and models of the relationship of pollutants to visibility have been devised⁸. Visibility measurements themselves have limitations as indicators of pollution levels, because of the effects of humidity, winds and other atmospheric conditions.

Fig. 2 Points with steady increases or decreases in the number of hazy hours for the period 1953–72. The Spearman rank correlation test was used. Others, not showing a steady trend, are excluded. ▲, An increase; ●, a decrease.



The difficulties have been carefully discussed by Miller *et al.*⁹ and Charlson¹⁰.

There are several ways of evaluating any such trends. To take into account how humans, rather than geographical areas, are affected by changes in visibility, we have weighted the results for each city by its metropolitan population. This was done in computing the points in Fig. 1. The 17 cities included comprise, as of the last census, about half the national population. Other ways of calculating average visibility, for example by dividing observation hours by cumulative miles of visibility (excluding hours in which precipitation occurred), give very similar results to Fig. 1. (ref. 11).

Figure 1 indicates that for major Canadian cities, the visibility tended to improve from 1953 to about 1963, when it tended to level off. A pair of lines of regression, both with 1963 as an end point, were calculated. The 1953–63 line had a slope about six times that of the later period.

To find effects on visibility over a wide area, rather than that in cities, we have considered the results of all 71 stations, most of which are not near large cities. For analysis, the Spearman rank correlation method was used, with the annual number of hazy hours compared year by year. Those stations whose change was statistically significant at the 0.05 level are shown in Fig. 2; others are excluded. Large areas, relatively less populated, show a steady increase in hazy days. While some large cities and one northern station have apparently increased their visibility over the two-decade period, the nation as a whole has not shown this trend.

While the visibility at major Canadian cities improved over the first decade of the period considered, there was little or no improvement in the second decade. The explanation for the left-hand portion of Fig. 1 is probably the replacement of coal with less obviously polluting fuels like oil and natural gas; the same phenomenon has been observed in the UK¹² and elsewhere. The post-1963 results are probably caused by a combination of the increased use of all fuels and slight decreases in the amount of pollutants per unit of energy produced. These changes have occurred without national laws on the subject, in contrast to the UK.

The fact that 24 stations of the total of 71 showed a steady increase in haze indicates that visibility outside the metropolitan areas is not improving. This may arise from a worldwide decrease in visibility of an order too small to show up in Fig. 1. The difference between the metropolitan and non-metropolitan trends may explain divergent opinions^{13,14} about similar atmospheric data.

The changes described here can induce significant climatic changes. In turn, climatic changes may produce alterations in visibility. By using similar data, we may eventually be able to judge the relationship of world visibility changes to the world climate.

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H. INHABER

Advanced Concepts Centre,
Environment Canada,
Ottawa, Canada, K1A 0H3

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- ¹ Mitchell, J. M., *Climatic Change* (World Meteorological Organization Technical Note No. 79, Geneva, 1966).
- ² *Causes of Climatic Change*, (edit. by Mitchell, J. M.) (American Meteorological Society, Boston, 1968).
- ³ Hare, F. K., and Thomas, M. K., *Climate Canada*, 97 (Wiley, Toronto, 1974).
- ⁴ Munn, R. E., and Bolin, B., *Atmos. Environ.*, **5**, 363–402 (1971).
- ⁵ Healy, T. V., *Atmos. Environ.*, **8**, 81–83 (1974).
- ⁶ Koike, S., *et al.*, *Proc. 13th Symp. Jap. Soc. Air Poll.*, Nov. 7–9, 249 (1972).
- ⁷ Koike, S., and Inoue, M., *J. Jap. Soc. Air Poll.*, **8** (3), 517 (1973).
- ⁸ Mitchell, J. M., *J. appl. Meteorol.*, **10**, 703–714 (1971).
- ⁹ Miller, M. E., Canfield, N. L., Ritter, T. A., and Weaver, C. R., *Mon. Weather Rev.*, **100**(1), 67–71 (1972).
- ¹⁰ Charlson, R. J., *Environ. Sci. Technol.*, **3**, 913–918 (1969).
- ¹¹ Inhaber, H., *Science*, **186** (4166), 798–805 (1974).
- ¹² Parker, A., *Clean Air*, **4**(4), 24–26 (1974).
- ¹³ Bryson, R. A., and Wendland, W. M., in *Global Effects of Environmental Pollution* (edit. by Singer, S. F.), 130–138 (Reidel, Holland, 1970).
- ¹⁴ Mitchell, J. M., in *Global Effects of Environmental Pollution* (edit. by Singer, S. F.), 139–155 (Reidel, Holland, 1970).

Light-scattering experiments on water vapour at pressures approaching saturation

THE existence of dimers in water vapour at pressures approaching saturation has been postulated for some years and a number of observations have been interpreted in terms of them¹⁻⁴. The relative dimer concentration is expected to increase sharply with increasing percentage saturation and decreasing temperature^{1,3}. From speed of sound measurements, a dimer concentration of 8 parts per 10³ has been reported at 50 °C and 90% saturation³; this might be expected to increase to >20% at 95% saturation and 20 °C. It may be supposed that the polarisability of the water dimer will be considerably larger than that of the monomer, and therefore, using an assumed value for polarisability, an estimate of the dimer concentration at a given temperature and vapour pressure can be made by comparing the quadratic term in a plot of the light scattered as a function of pressure with the known second virial coefficient. In early experiments, using a large multi-pass scattering cell, a very large quadratic term was observed. This arose, however, largely from a rapid increase in the elastic scattering from water-contaminated surfaces as the vapour pressure in the cell approached saturation. We have now eliminated this spurious scattering by selecting spectroscopically only those photons which exhibit a frequency shift due to Brillouin and Raman scattering.

In these experiments two particular difficulties were found: elimination of parasitic light by spectral analysis leads to a large reduction of an already weak signal, and the peculiar problem of reliable pressure measurement with water vapour. Because of surface adsorption, the pressure changes considerably after charging a system with vapour. It was found that long periods, often >1 h, were required before 1% stability could be established in our cell. The consequently long experimental periods thus necessitated very good stability of optics and laser power and constancy of detector response. (For a discussion of high precision photon counting applied to light-scattering studies see ref. 5.)

In our experiments the beam from an argon ion laser (300 mW; 488.0 nm) was directed through a small cell, containing variable amounts of water vapour, and light scattered at 90° to the beam (containing Raman, Brillouin and Rayleigh components) was detected after passing through a confocal, piezo-electrically scanned, Fabry-Perot etalon of free spectral range 375 MHz and finesse ~ 60. The photon counts were registered in a multi-channel analyser. Frequency-shifted light,

scattered from the vapour, was selected by counting only photons between Rayleigh peaks. A reference beam, using the same collection system and detector served as a monitor from which small corrections could be made for fluctuations of laser power or detector sensitivity.

A representative set of results is shown in Fig. 1 with a least-squares quadratic fit to all data points up to and including the cluster of four observations close to 95% saturation vapour pressure (SVP). The parabola is of the form $S = aP + bP^2$ (where S = photon count and P = pressure) and we find the quadratic term (bP^2/aP) has the value of $2.2 \pm 1 \times 10^{-3}$ at 95% SVP. Values of the second virial coefficient B in water vapour, and its division between a van der Waals' component B_1 and a 'dimer' component B_2 , have been given in ref. 4. These suggest that at 95% SVP and 20 °C, $\sim 0.45 \times 10^{-3}$ of the quadratic scattering can be attributed to the increased concentration of monomers because of the van der Waals' attraction (B_1); the remainder $1.75 \pm 1 \times 10^{-3}$ must be attributed to scattering from dimers. The assumption that scattering from a dimer is twice that from the monomer leads to a fractional concentration of dimers of $0.88 \pm 0.5 \times 10^{-3}$ —an order of magnitude less than the earlier reported values³. On the other hand, the values of B_2 given in ref. 4 would suggest a fractional dimer concentration of only 0.82×10^{-3} at 20 °C and 95% SVP—a value supported by our light-scattering measurements. Alternatively, by adopting this latter value for the concentration, we can calculate the scattering from a dimer as 2.2 times that from a monomer—a figure which is probably quite reasonable in consideration of the likely polarisability of a linear or chain-like dimer⁷. Our experiment consisted of a search for small effects in quasi-elastic scattering, and its accuracy is limited by the peculiar problems of working with water vapour. It seems likely that a Raman scattering experiment should be more definitive, with the possibility of associating specific spectral features with the water dimers.

In conclusion, we note that Fig. 1 shows a number of readings at, and very close to, SVP. In these observations it is likely that significant condensation occurred in the cell, and that small (<0.2 °C) temperature fluctuations during each experiment contributed significantly to small pressure variations. Owing to the possibility of water droplet formation on the windows these results must therefore be interpreted with caution in spite of our monitoring technique. One might, however, expect relatively large concentrations of dimers and multimers at SVP and, as a consequence, the increased scattering which is observed.

K. BURROWS

SRC, Appleton Laboratory,
Ditton Park, Slough, UK

E. R. PIKE
J. M. VAUGHAN

Royal Radar Establishment,
Malvern, UK

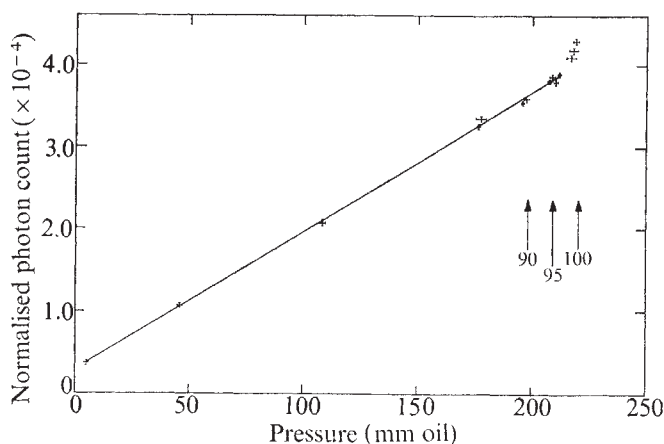
Received August 14, 1975; accepted January 28, 1976.

- Gebbie, H. A., Bohlander, R. A., and Pardoe, G. W. F., *Nature*, **230**, 521 (1971).
- Bohlander, R. A., Gebbie, H. A., and Pardoe, G. W. F., *Nature*, **228**, 156 (1970).
- Ashwell, G. E., Eggett, P. A., Emery, R., and Gebbie, H. A., *Nature*, **247**, 196 (1974).
- Bohlander, R. A., and Gebbie, H. A., *Nature*, **253**, 523 (1975).
- Pike, E. R., Pomeroy, W. R. M., and Vaughan, J. M., *J. chem. Phys.*, **62**, 3188 (1975).
- Greytak, T. J., and Benedek, G. B., *Phys. Rev. Lett.*, **17**, 179 (1966).
- Water*, 1 (edit. by Franks, F.), 101 and 372 (Plenum, New York, London, 1972).

Major magmatic activity as a key to predicting large earthquakes along the Sagami Trough, Japan

MIHARA-YAMA is a basaltic stratovolcano on Oshima, an island lying about 20 km off the coast of Izu Peninsula in central Japan (Fig. 1). I attempt here to show that the magmatic activity of Mihara-yama could be used to predict

Fig. 1 Variation of inelastic scattered light with water vapour pressure at 20.2 °C. A least-squares parabola (indistinguishable from a straight line at this scale) is fitted to the data points up to 95% SVP. Error bars indicate s.d. in photon counts and observed pressure variations. Arrows indicate % SVP.



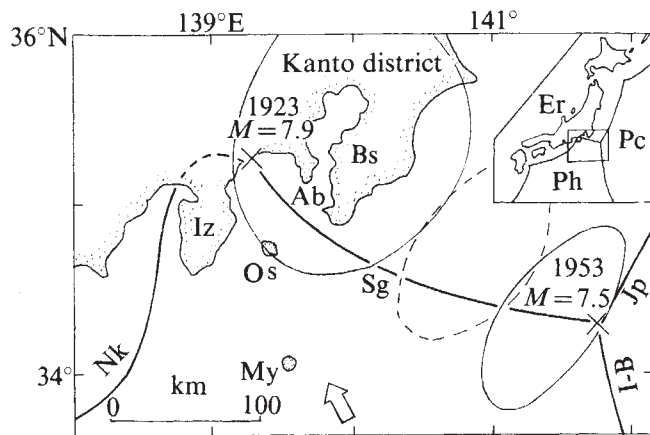


Fig. 1 Large earthquakes that have occurred along the Sagami Trough during this century. The aftershock area is enclosed within the solid line, and the seismicity gap is enclosed within the broken line (see ref. 6). $\times$, Epicentre of the large earthquake; arrow, direction of slip of the northern Philippine Sea Plate³; Sg, Sagami Trough; Nk, Nankai Trough; Jp, Japan Trench; I-B, Izu-Bonin Trench; Os, Oshima; My, Miyakejima; Ab, Aburatsubo; Bs, Boso Peninsula; Iz, Izu Peninsula. Inset: Ph, Philippine Sea Plate; Pc, Pacific Plate; Er, Eurasian Plate.

large earthquakes along the Sagami Trough. There is apparently a causal relationship between eruptions of Mihara-yama and the earthquakes.

The floor of the summit crater of Mihara-yama rose approximately 400 m before the Great Kanto Earthquake of 1923 ($M=7.9$) and the Bosoiki Earthquake of 1953 ($M=7.5$), (see ref. 1). Both earthquakes occurred along the Sagami Trough, which extends from the Japan Trench, 180 km south-east of the Boso Peninsula, through Sagami Bay to the city of Odawara on the coast (Fig. 1).

The Sagami Trough forms a border between the Philippine Sea Plate and the Eurasian Plate (ref. 2 and Fig. 1). It is a right-lateral fault with an underthrusting component³ indicating subduction of the Philippine Sea Plate. Stress between the two plates causes seismic activity along the trough. I have noticed that subsidence of the southern

Kanto district around Aburatsubo occurs concurrently with a north-westerly movement of the Philippine Sea Plate. Marked drops in the altitude of the area were recorded before the earthquakes of 1923 and 1953. Further, on both occasions, as the land mass dropped, the floor of Mihara-yama's summit crater rose as much as 400 m, and the volcano erupted.

The earthquakes occurred almost concurrently. The crater's floor fell when the Aburatsubo area rose again after the earthquakes (Fig. 2). It has been suggested that the magma is squeezed up by the compressional crustal strain¹. I conclude therefore, that the increased compressional crustal stress along the trough forces a downward movement of the Aburatsubo area and squeezes up the magma beneath Mihara-yama, and that, consequently, large earthquakes occur to release strain along the trough. Such activity is regarded as one contributing factor to a major eruption and earthquake.

Mihara-yama's volcanic activity is apparently related to that of Miyakejima, a volcanic island about 80 km south of Oshima. When crustal stress builds up along the trough, Miyakejima begins a cycle of activity of volcanic earthquakes with three frequency maxima, erupting during the second maximum (Fig. 3). Volcanic earthquakes result as stress concentrates beneath the smaller volcano. The stress, though not strong enough to trigger a major eruption of Mihara-yama, does stimulate activity beneath it. In fact, when Miyakejima erupts, a minor eruption occurs on Mihara-yama. When Mihara-yama erupts, all volcanic activity on Miyakejima stops. This implies that strain is first released on Miyakejima, and then accumulates under Mihara-yama. This is a major contributing factor in causing Mihara-yama to enter the active stage. Miyakejima last erupted in 1962. Mihara-yama began its first maximum in 1970; it will probably end sometime in 1975 (Fig. 3).

During the past 200 yr, four large eruptions have occurred on Miyakejima, followed by large eruptions on Mihara-yama at intervals ranging from 2 to 17 yr after activity was first noted on Miyakejima. Large earthquakes followed the eruptions of Mihara-yama within 3–11 yr (ref. 4). Since Miyakejima erupted in 1962, and since Mihara-yama has almost completed its first maximum, we can safely assume

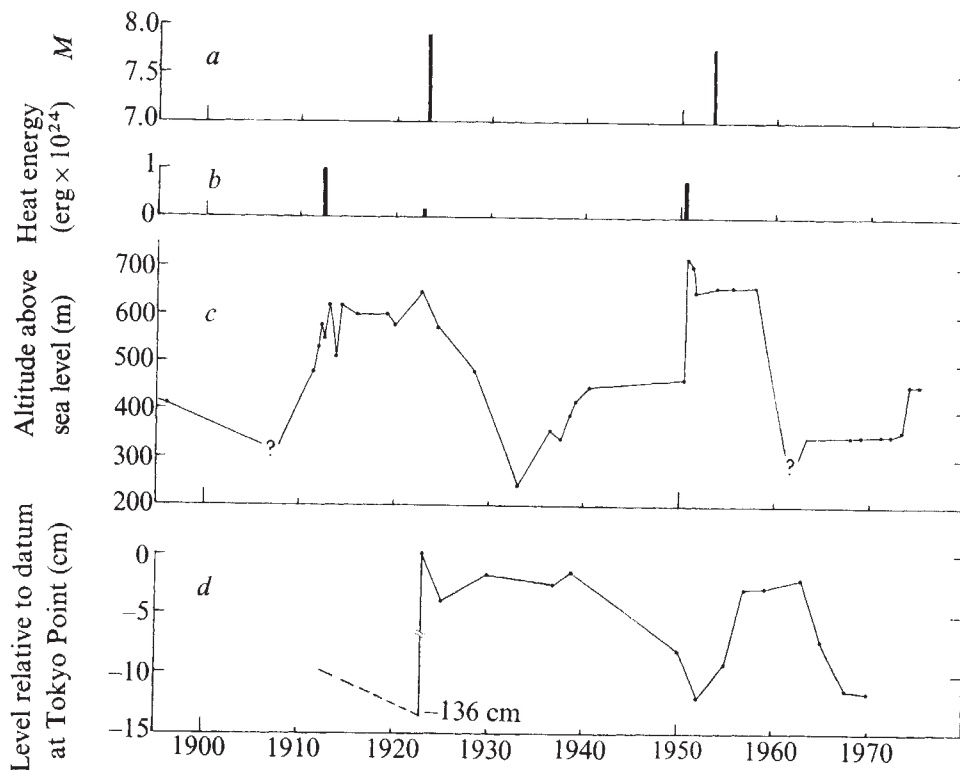
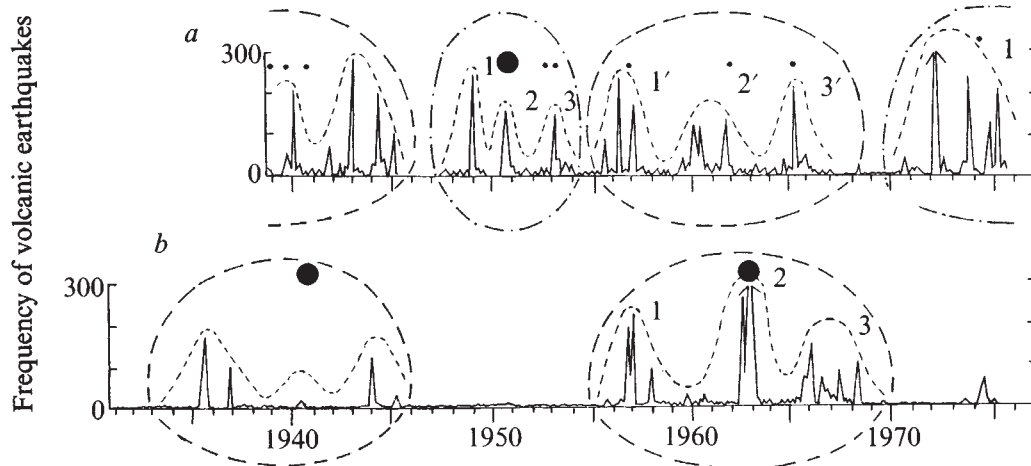


Fig. 2 Relationship between large eruptions, changes in level of floor of summit crater of Mihara-yama, and large earthquakes that have occurred along the Sagami Trough. *a*, Record of large earthquakes along the Sagami Trough (used magnitude data were officially determined by the Japan Meteorological Agency); *b*, heat energy discharged by large eruptions of Mihara-yama¹; *c*, changes in levels of floor of the summit crater of Mihara-yama (ordinate shows the height above sea level; levelling data to 1958 from refs 7 and 8, after 1958, collected by author); *d*, changes in level at Aburatsubo (solid line measured with precise levelling relative to datum at Tokyo Point⁹, and the broken line was deduced from tidal data¹⁰).

Fig. 3 Relationship between volcanic earthquakes and eruptions in Oshima (a) and Miyakejima (b) (data from the Japan Meteorological Agency). ●, Eruption points (large dots represent large eruptions; smaller dots represent smaller eruptions which ejected essential magmatic materials). Portions enclosed in the regular broken line, active stages of Miyakejima (b). Correlative activity at Oshima (a), (relative to datum at Miyakejima) is also enclosed in a regular broken line. Portions enclosed in the irregular broken line (-----), show active stage of Mihara-yama, Oshima. 1, 2, 3 and 1', 2', 3', maxima of frequency of volcanic earthquakes in respective stages.



that it will erupt sometime between 1975 and 1979. In fact, in 1974 I observed a 100-m elevation of the floor of Mihara-yama's summit crater (ref. 5 and Fig. 2c).

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MASAAKI KIMURA

Geological Survey of Japan,
Hisamoto, Kawasaki, Japan

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- ¹ Nakamura, K., *Bull. volcan. Soc. Japan*, **16**, 63-71 (1971).
- ² Sugimura, A., *Kagaku*, **42**, 192-202 (1972).
- ³ Kanamori, H., and Ando, M., *Publs 50th Anniversary of the Great Kanto Earthquake, 1923*, 89-101 (Earthquake Research Institute, University of Tokyo, 1973).
- ⁴ Kimura, M., *J. Geogr.*, **82**, 171-188 (1973).
- ⁵ Kimura, M., and Toyoda, J., *Bull. volcan. Soc. Japan*, **19**, 65-78 (1975).
- ⁶ Nagumo, S., *Publs 50th Anniversary of the Great Kanto Earthquake, 1923*, 273-291 (Earthquake Research Institute, University of Tokyo, 1973).
- ⁷ Tsuya, H., Okada, A., and Watanabe, T., *Bull. Earthq. Res. Inst., Tokyo Univ.*, **34**, 33-59 (1956).
- ⁸ Kizawa, T., and Tanaka, Y., *Pap. Meteorol. Geophys.*, **23**, 411-428 (1972).
- ⁹ Fujita, N., *Rep. coord. Comm. Earthquake Prediction*, **5**, 36-37 (1971).
- ¹⁰ Imamura, A., *Publs Imp. Earthq. invest. Comm.*, **25**, 1-143 (1930).

the high temperature peak remained prominent after 14 d at 76 °C, when ~ 40% of the total TL still remained. The fading of all MgO peaks was considerable (Fig. 2) where samples read out after intervals of 25 h, 9 d and 6 weeks are compared with a LiF TLD-100 extruded chip readout after 6 h. Nevertheless, the previously unreported

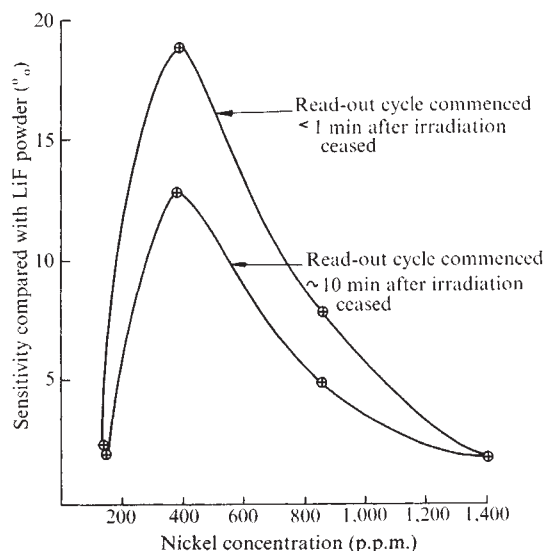
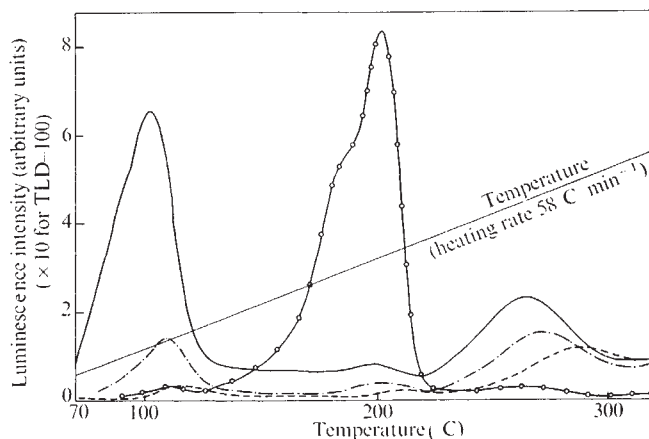


Fig. 1 Variation with concentration of Ni in TL sensitivity of MgO-Ni relative to LiF.

Fig. 2 Glow curves for TLD-100 (chip) and MgO: 370 p.p.m. Ni (chip) exposed to 63 r. ⁶⁰Co radiation. —○—○—, LiF after 6 h; —, MgO after 25 h; - - - - -, MgO after 9 d; ————, MgO after 6 weeks.



Thermoluminescence of magnesium oxide

MAGNESIUM oxide containing impurity levels of 130-1,400 p.p.m. Ni was investigated for use as a γ -radiation dosimeter, and compared with the widely used LiF (TLD-100). Other samples of MgO containing B, Cr, Fe and Co were also tried but none possesses the required characteristics. It has previously been shown¹ that MgO is suitable for neutron dosimetry when thermally stimulated exoelectrons (TSEE) are used.

The atomic number of MgO is relatively high by comparison with LiF, but this is useful since if the two materials were used in conjunction, it would provide a means of energy discrimination. MgO is also relatively cheap.

Samples of all the MgO types were first exposed to 1 kr. of ⁶⁰Co γ radiation, and the thermoluminescence (TL) colour observed by eye. A blue colour, suitable for matching the response of the photomultiplier tube in use, was only obtained from Ni-activated samples. Glow curves were then obtained after exposure to 100 r. of the same radiation². The most marked difference between the glow curve of MgO containing Ni and that of pure MgO (ref. 3) and MgO containing other impurities⁴, is the presence of a glow peak from 270 °C-320 °C; the peak tends towards the lower temperatures as the exposure is increased. The integrated TL from 75 to 325 °C was obtained for the complete range of nickel impurity concentration and indicated that 370 p.p.m. was the optimum value (Fig. 1). This material was found to have a linear response over the exposure range of 100 r.-100 kr. It was further found that

high temperature peak in MgO-Ni could be of use for high levels of radiation exposure measurement. The kinetics of the glow curves, energy level values and luminescence spectra are being investigated.

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A. C. CARTER

Rutherford Laboratory,
The Royal Military College of Science,
Shrivenham, Swindon,
Wiltshire SN6 8LA, UK

Received July 7, 1975; accepted January 28, 1976.

¹ Ritz, V. H., and Attix, F. H., *Appl. Phys. Lett.*, 23(3), 166-167 (1973).

² Carter, A. C., thesis, Univ. Surrey (1974).

³ Nanto, H., et al., *J. Phys. Chem. Solids*, 36, 477-478 (1975).

⁴ Ziniker, W. M., Rusin, J. M., and Stoebe, T. G., *J. Mat. Sci.*, 8, 407 (1973).

Influence of magnesium on nickel toxicity

THE influence of calcium in ameliorating heavy metal toxicity towards plants is well known. The possibility that magnesium might possess similar properties, if to a lesser extent, has been largely ignored by ecologists although there are indications in the literature that such is the case^{1,2}. We here reaffirm the effect, and point out its ecological significance.

We investigated the growth of oats (*Avena sativa* L. var. Selma) over a wide range of nickel and magnesium concentrations in water culture. We here report in detail one of our experiments in which oats were grown at magnesium levels of 1.25, 2.5, 5.0 and 7.5 mM combined with nickel at 0 and 1.5 p.p.m. The oats were pregerminated and transferred as seedlings to containers filled with 1.6 l of culture solutions. The culture solutions contained, in addition to the experimentally varied levels of MgSO₄ and Ni(NO₃)₂, 1 mM NH₄H₂PO₄, 6 mM KNO₃, 0.5 mM Ca(NO₃)₂, 7 mM NaNO₃, 0.1 mM NaCl, and, as micronutrients, 90 µM NaFeEDTA, 46 µM HBO₃, 9.1 µM MnSO₄, 0.76 µM ZnSO₄, 0.32 µM CuSO₄, 9.1 µM (NH₄)₆Mo₇O₂₄. The pH was adjusted to 5.2 at each weekly change of culture solution.

The experimental layout was of a randomised block design; there were 64 containers in all, each with three oat plants, with 8 blocks for each treatment. The experiment was run for 35 d in a shaded glasshouse with 18 h d⁻¹ supplementary light. Individuals were then scored for Ni toxicity symptoms and harvested. They were dried at 75 °C, weighed on a per pot basis, and the results (Table 1) subjected to an analysis of variance which indicated significant main effects of both Ni ($P < 0.001$), Mg ($P < 0.05$) and Ni-Mg interaction ($P < 0.025$).

Figure 1 shows that the characteristic Ni toxicity symptoms (described in detail by Vergnano and Hunter³) decreased in severity with increasing Mg concentrations. The dry weight measurements (Table 1) showed no significant difference between the oats grown at 0 p.p.m. Ni and 1.25, 2.5 and 5.0 mM Mg. The fall in yield at the 7.5 mM level is presumed to result from an excess of Mg. In contrast, there were marked

increases in weight over the range 1.25-5.0 mM Mg at 1.5 p.p.m. Ni. This experiment, and a number of others not reported in detail here, show that Ni toxicity can be ameliorated by increasing Mg levels. (We have no evidence that sulphate anions influence Ni toxicity over the concentration ranges used.)

A further aspect of the influence of Mg on Ni toxicity was shown in an experiment using very high levels of magnesium (25 and 40 mM) with high levels of nickel (5 and 10 p.p.m.). At 40 mM magnesium the plants were very badly stunted, but showed no symptoms of the toxicity of Ni, and were indistinguishable from plants subjected to the same high level of Mg with no Ni. At 25 mM Mg, the toxicity symptoms of Ni were completely suppressed at the 5 p.p.m. Ni level, but were visible to a small extent at the 10 p.p.m. level.

Further work is in progress to investigate the means by which Mg ameliorates Ni toxicity, but it seems worthwhile to comment on the ecological significance of these preliminary observations. Serpentine soils very often contain high levels of

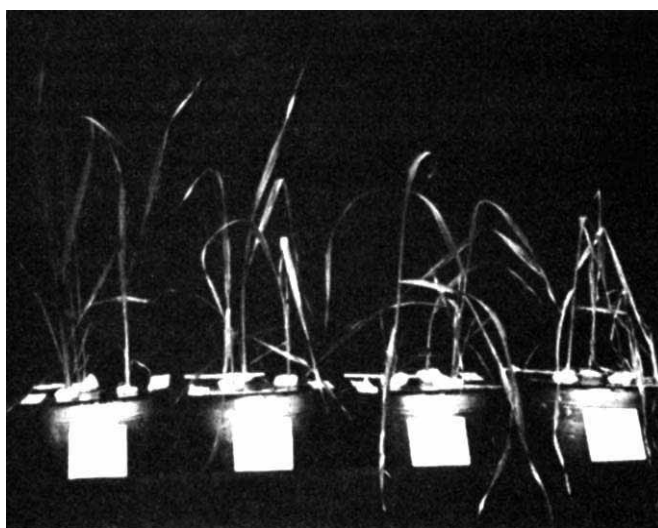


Fig. 1 Increasing symptoms of nickel toxicity in oats with decreasing levels of magnesium (from left to right: 7.5; 5.0; 2.5; and 1.25 mM).

Mg and relatively large quantities of Ni, and many authors have used oats as a bioassay for the presence of toxic levels of Ni in these soils^{4,5}. The failure to detect Ni toxicity in a number of such soils (ref. 4 and J.P., unpublished) in spite of evidence that appreciable quantities of Ni were present, may arise partly from the influence of high levels of Mg. The use of oats as a bioassay for Ni toxicity can now be seen to involve an oversimplification of the soil situation and assumes that the Ni-Mg interaction is similar for other species.

The response to Mg of other Ni-sensitive species remains to be investigated, but we feel a consideration of Ni-Mg interactions must be involved in future studies on serpentine soils.

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JOHN PROCTOR
IAIN D. MCGOWAN

Biology Department,
University of Stirling,
Stirling, UK

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¹ Abelson, P. H., and Aldous, E., *J. Bact.*, 60, 401-413 (1950).

² Crooke, W. M., and Inkson, R. H. E., *Pl. Soil*, 6, 1-15 (1955).

³ Vergnano, O., and Hunter, J. G., *Ann. Bot.*, 17, 317-328 (1953).

⁴ Proctor, J., *J. Ecol.*, 59, 397-410 (1971).

⁵ Williams, P. C., *Nature*, 214, 628 (1967).

Table 1 Mean dry weight $\pm$ s.e. (g) of oat plants grown in culture solutions with differing magnesium and nickel levels. We have, for brevity, combined root and shoot data: their response is similar, although the Ni-Mg interaction is more marked for the roots

Mg (mM)	Ni (p.p.m.)	
	0	1.5
1.25	2.01 $\pm$ 0.19*	0.83 $\pm$ 0.17
2.50	1.99 $\pm$ 0.17	1.21 $\pm$ 0.17
5.00	2.02 $\pm$ 0.17	1.62 $\pm$ 0.17
7.50	1.38 $\pm$ 0.17	1.35 $\pm$ 0.17

*Mean of seven observations only.

Interoceanic differences in vulnerability of shelled prey to crab predation

SHELLED gastropods living on intertidal open rocky surfaces exhibit various shell features which can be interpreted as adaptations against such shell-breaking predators as fishes, spiny lobsters, and crabs. These anti-predatory devices include strong external shell sculpture, elongate or dentate apertures and low spires¹. Not only are these characteristics more common among tropical species compared with ecologically similar cold-water forms, but within the tropics they are more prevalent and better developed in the Pacific and Indian Oceans than in the Atlantic^{1,2}. Any or all of three conditions must obtain in areas with a high incidence and strong development of anti-predatory armour compared with areas where such armour is reduced: the prey are stronger, that is, the criteria needed by the prey to avoid being crushed are more stringent; the predators are stronger; and shell-breaking predators account for relatively greater mortality. Here I report data confirming the first two of these possibilities.

At the University of Guam Marine Laboratory (Pago Bay, Guam, Mariana Islands), various reef-flat crabs (Table 1) were found to feed readily on shelled gastropods and hermit crabs by crushing them in their massive master claw. Individual crabs were kept in aquaria with aerated or recirculating seawater. The critical size (maximum size of vulnerability) of prey was obtained for individual crabs by offering them a range of sizes of that prey species. The common occurrence in the field of snails whose shells show repair after presumed damage by a predator suggests that shell breakers often attack prey that are too large for them.

Direct comparisons between Guamanian and ecologically similar West Indian prey were made by allowing hermit crabs to inhabit Jamaican shells, which were then offered to the predatory crabs. Shells were collected alive near Discovery Bay, Jamaica; the soft parts were removed and the shells dried in air before being used in Guam 4–9 months later. Shells treated in this way seem to possess mechanical properties indistinguishable from those of living snails³.

Although in most cases not enough shells were at hand to

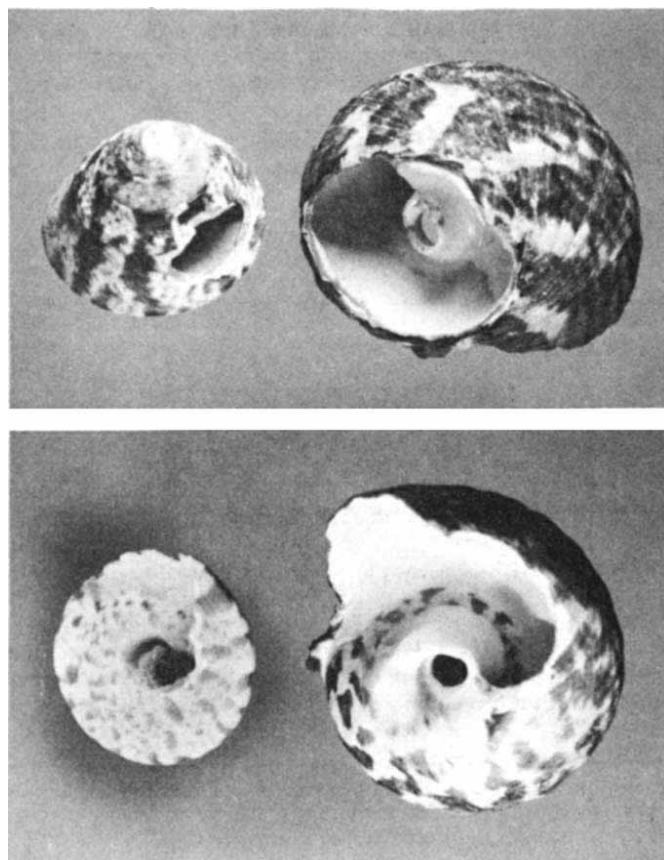


Fig. 1 Shells of the Jamaican *Cittarium pica* (left) and Guamanian *Trochus niloticus* (right) broken by the Pacific crab *Carpilius maculatus*. The two views of each species pertain to the same shell. Photograph by F. Dixon.

establish the critical size of Jamaican prey, it is evident from Table 1 and Fig. 1 that this critical size is greater than that of ecologically equivalent and taxonomically related Guamanian snails. Since the largest offered specimens of all West Indian species were extensively damaged, it is likely that the critical

Table 1 Critical sizes and shell thicknesses of Guamanian (G) and Jamaican (J) shelled prey crushed by Guamanian predatory crabs

Crab	Species	Width (mm)	Species	Prey Critical size (mm)	Largest size offered (mm)	Shell thickness (mm)
<i>Carpilius maculatus</i>	(G) <i>Trochus niloticus</i>	115.3		39.5	49.0	1.3–2.5
	(J) <i>Cittarium pica</i>			57.4	57.4	1.1–3.8
	(G) <i>T. niloticus</i>	138.1		39.5	45.1	—
<i>Carpilius convexus</i>	(J) <i>C. pica</i>			55.3	55.3	—
	(G) <i>T. niloticus</i>	89.9		37.0	38.2	—
	(J) <i>C. pica</i>			48.2	48.2	—
<i>Daldorfia horrida</i>	(G) <i>T. niloticus</i>	82.8		35.0	42.0	—
	(J) <i>C. pica</i>			38.0	38.0	—
<i>Eriphia sebana</i>	(G) <i>T. niloticus</i>	65.3		25.3	31.1	—
	(J) <i>C. pica</i>			34.0	34.0	—
	(G) <i>Conus spp.</i>	115.3		30.5	37.2	1.4–2.8
<i>Daldorfia horrida</i>	(J) <i>Conus mus</i>			39.7	39.7*	1.1
	(G) <i>Conus spp.</i>	82.8		31.3	40.3	—
	(J) <i>Conus mus</i>			32.5	32.5	—
<i>Eriphia sebana</i>	(G) <i>Nerita albicilla</i>	56.1		23.6	24.3	1.1–1.9
	(J) <i>N. tessellata</i>			20.9	20.9*	0.8–1.5
	(G) <i>N. albicilla</i>	65.3		27.1	30.0*	—
	(J) <i>N. tessellata</i>			20.7	20.7*	—
	(G) <i>N. plicata</i>	56.1		21.0	22.6	1.3–1.7
	(J) <i>N. versicolor</i>			21.7	21.7	0.9–1.0
	(J) <i>N. peloronta</i>			30.0	30.0	0.8–1.0
	(G) <i>N. plicata</i>	65.3		27.9	27.9*	—
	(J) <i>N. versicolor</i>			27.2	27.2*	—
	(J) <i>N. peloronta</i>			34.5	34.5	—

*Close to, or equal to, maximum adult size.

Critical sizes for Guamanian prey are defined as the mean between the largest specimen crushed and the next larger one which remained intact or which sustained only minor damage to the outer lip. These two sizes were never more than 1.5 mm apart.

Table 2 Vulnerability of *N. lapillus* to predation by Guamanian (G) and Irish (I)^{3,4} predatory crabs

Crab species	Width (mm)	Thick high-spired form Largest crushed (mm)	Largest given (mm)	Thin low-spired form Largest crushed (mm)	Largest given (mm)
(G) <i>Carpilius maculatus</i>	105.4 111.3 115.3	29.0 29.4 33.4	29.0 29.4 33.4*	— — —	— — —
(G) <i>Carpilius convexus</i>	89.9	27.2	27.2	—	—
(G) <i>Daldorfia horrida</i>	82.8	32.8	32.8	—	—
(G) <i>Eriphia sebana</i>	56.1	27.5	27.5	—	—
(I) <i>Portunus puber</i>	80	20	—	25	—
(I) <i>Carcinus maenas</i>	59 66 75	22 12 12	24 27 27	24 25 25	27 27 29

*Close to maximum adult size in Maine.

sizes of these species are markedly larger than the maximum sizes available in my experiments. Even so, the largest successfully crushed *Cittarium pica* L. are about 1.5 times as large in body-whorl diameter as the Guamanian analogue, *Trochus niloticus* L. The superiority of *Trochus* over *Cittarium* stems in part from its relatively smaller umbilicus and proportionately smaller extent of outer shell wall not supported from within by earlier whorls (Fig. 1). *T. niloticus* also attains a larger adult size (150 mm compared with 110 mm in *C. pica*).

both components of cross-sectional area relative to carapace width are greater among Indo-West-Pacific taxa than in their Caribbean counterparts (Table 3). No differences in manus height or manus thickness could be detected between males and females of either *Carpilius maculatus* or *C. convexus*, but in the other species (Table 3) the manus of the males is higher and thicker than that of the females. Differences between species are preserved even when only females or only males are compared. I found no differences in claw form between different

Table 3 Relative manus height and manus thickness in the Indo-West-Pacific (P) and Western Atlantic (A) species of *Carpilius* and *Eriphia**

Species	Carapace width (mm)	Manus height		Manus thickness		n
		Carapace width		Carapace width		
		Mean	Range	Mean	Range	
(P) <i>Carpilius maculatus</i> L.	63.6–138.1	0.422	0.345–0.495	0.245	0.223–0.260	14
(P) <i>C. convexus</i> Forskal	56.1–90.1	0.386	0.358–0.424	0.234	0.211–0.261	15
(A) <i>C. corallinus</i> Herbst	61.3–142.3	0.355	0.327–0.406	0.211	0.202–0.247	11
(P) <i>Eriphia sebana</i> Shaw and Nodder	36.7–65.3	0.503	0.411–0.605	0.314	0.262–0.366	23
(P) <i>E. smithii</i> McLeay	34.9–65.1	0.475	0.418–0.504	0.315	0.269–0.371	8
(P) <i>E. scabricula</i> Dana	21.0–24.4	0.440	0.414–0.463	0.308	0.299–0.314	4
(A) <i>E. gonagra</i> Fabricius	18.8–43.7	0.416	0.373–0.485	0.288	0.254–0.344	17

*All intrageneric differences are significant at the 0.01 level or better (Mann-Whitney *U* test) except that for manus thickness between *E. sebana* and *E. smithii*. *E. scabricula* was not statistically compared with other species, and is included only for completeness.

The Jamaican *Conus mus* Hwass is thinner-walled (Table 1), higher-spined, and more broadly apertured than any Guamanian *Conus* normally found on hard substrata (*C. chaldaeus* Röding, *C. coronatus* Gmelin, *C. ebraeus* L., *C. flavidus* Lamarck, *C. lividus* Hwass, *C. miles* L., *C. sponsalis* Hwass). Among high intertidal species of *Nerita*, the West Indian *N. peloronta* L. and *N. versicolor* Gmelin are thinner-shelled and more vulnerable to predation by the intertidal crab *Eriphia sebana* than the Guamanian *N. plicata* L. (Table 1). The lower intertidal *N. tessellata* Gmelin from Jamaica is smaller and more vulnerable than, but comparable in shell thickness with, its Guamanian analogue *N. albicilla* L. (Table 1).

In other trials, individual crabs were offered hermit specimens of the cold-temperate snail *Nucella lapillus* L., whose North Atlantic crab predators (*Portunus puber* L., *Carcinus maenas* L. and *Cancer pagurus* L.) have been well studied in Ireland^{4,5}. Thick-shelled high-spined specimens of *Nucella* were collected in August 1971, from sheltered sites near Boothbay Harbor, Maine. Table 2 suggests that Guamanian crabs can crush larger *Nucella* and are more powerful than similarly-sized Irish crabs.

Among the factors affecting the strength of a crab's master claw is the cross-sectional area of muscle in the manus (that part of the propodus behind the opposing fingers). Both components of cross-sectional area, manus height (dorsiventral dimension) and manus thickness (anteroposterior dimension), were measured on crabs in the US National Museum, Washington, DC. Within the genera *Eriphia* and *Carpilius*,

populations of the same species, and there seems to be no allometry of either manus height or manus thickness with increasing carapace width.

In summary, the arms race between Indo-West-Pacific rocky-shore gastropods and their crab predators has progressed further or remains in a more escalated state than in other tropical or temperate regions. Presumably, this high degree of coadaptation is related in part to the great age and stability of the tropical Pacific and Indian Oceans. The applicability of this finding to gastropods in other habitats, and to shell-breaking predators other than the xanthid and parthenopid crabs considered here, remains to be determined.

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GEERAT J. VERMEIJ

Department of Zoology,
University of Maryland,
College Park, Maryland 20742

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¹ Vermeij, G. J., *Evolution*, 28, 656–664 (1974).

² Vermeij, G. J., *J. mar. Res.*, 32, 343–357 (1974).

³ Curry, J. D., *J. mar. Biol. Assoc. U.K.*, 55, 419–424 (1975).

⁴ Ebling, F. J., Kitching, J. A., Muntz, L., and Taylor, C. M., *J. Anim. Ecol.*, 33, 73–83 (1964).

⁵ Kitching, J. A., Muntz, L., and Ebling, F. J., *J. Anim. Ecol.*, 35, 113–126 (1966).

Mechanism for digestion of detrital bacteria by the cichlid fish *Sarotherodon mossambicus* (Peters)

ALTHOUGH many species of fish ingest large amounts of detritus, its nutritional value has been questioned frequently^{1,2}. Detritus in marine and freshwater habitats consists of a mixture of plant debris and amorphous organic matter together with associated heterotrophic and autotrophic microorganisms³. In detritus which does not contain large amounts of undegraded vascular plant matter, most of the protein is associated with heterotrophic microorganisms³⁻⁸. This is particularly true in fresh water⁹. Thus, the value of detritus as a food for fish depends to a large extent on their ability to digest heterotrophic microorganisms. It has been suggested that fish which consume detritus may have this ability⁴. Using ¹⁴C-labelled bacteria in *in vitro* experiments I have now demonstrated it in *Sarotherodon mossambicus*.

Rapidly growing juvenile *S. mossambicus* (2.0–8.0 cm standard length) in Lake Sibaya (32°40'E, 27°25'S), Kwa-Zulu, feed entirely on benthic detritus which contains large numbers of diatoms, bacteria and amorphous organic material (my unpublished work). Gastric pH in specimens examined immediately after capture was measured with test papers (Acilit, Merck) read to 0.25 pH units. In stomachs of actively feeding fish, pH was consistently below 2.5, with values of 1.5 and 1.25 most common, and occasionally a pH of 1.0 was measured. Intestinal pH ranges from 8.0 to 8.8 (ref. 10). Residence time of material in the stomach is estimated to be about 1 h. Although gastric pH values below 2.0 are very unusual in vertebrates, Moriarty¹¹ found that the cichlid fish *Tilapia nilotica* in Lake George, Uganda, uses gastric acid below pH 2.0 to lyse blue-green algae. Because of the chemical similarity between bacterial and blue-green algal cell walls, it seemed likely that the unusually low gastric pH found in *S. mossambicus* would cause lysis of bacteria.

The effect of different gastric pH values on the extent of subsequent intestinal digestion of bacteria was investigated in *in vitro* simulations of digestion in *S. mossambicus*. Pooled stomach and pooled intestinal contents of ten actively feeding *S. mossambicus* were centrifuged and the supernatants decanted and stored at -10 °C until required. Protease activity in stomach juice was determined with a sensitivity of 2 µg pepsin per ml by the method of Anson and Mirsky¹². Detritus was collected from shallow sand terraces in Lake Sibaya, and bacteria in 20 ml of suspended detritus were labelled differentially with ¹⁴C-glucose (271 mCi mmol⁻¹) for 1 h at a concentration of 32.4 µg glucose per l. It has been shown that concentrations of glucose below 500 µg l⁻¹ are taken up by bacteria but are not high enough to be utilised by algae¹³. Unassimilated ¹⁴C label was removed by repeated suspension of the detritus sample in sterile water followed by centrifugation. The detritus was then resuspended in 20 ml of sterile water, and 3 ml of suspended detritus was transferred to each of six centrifuge tubes.

To simulate stomach conditions, hydrochloric acid was added to the detritus in five of the six centrifuge tubes to produce a range of pH values from 1.3 to 2.5. To compare acid and enzymatic lysis, lysozyme (2 mg in 2 ml of phosphate buffer, pH 6.2, from egg white, Sigma) was added to detritus in the sixth centrifuge tube. The tubes were then stoppered and placed horizontally in a shaking water bath at 25 °C. After 1 h, the six suspensions were centrifuged and the levels of radioactivity in 0.1 ml of each of the supernatants was determined by liquid scintillation counting. The remaining supernatant was discarded.

To simulate intestinal digestion, each detrital pellet was resuspended in 3 ml of intestinal juice at pH 8.6. Boiled

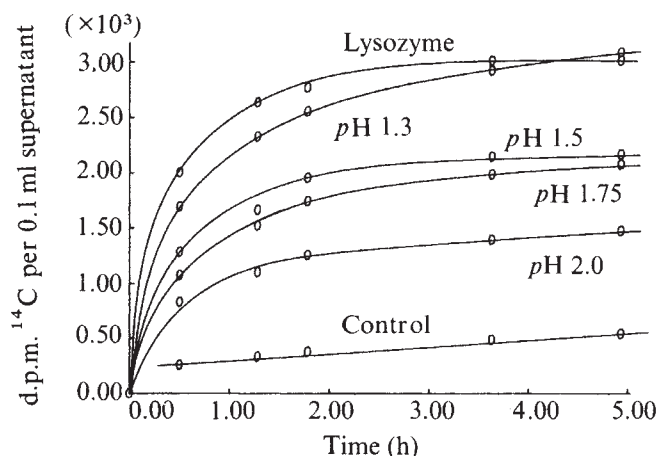


Fig. 1 Radioactivity released from bacteria during treatment with intestinal juice after acid treatment at pH values shown. Data given are experimental minus control with correction for volume reduction caused by sampling.

intestinal juice was used as a control. Release of ¹⁴C from bacteria was monitored by centrifuging the suspensions periodically and determining radioactivity in 0.1 ml of each of the supernatants. After sampling, the pellets were resuspended quickly and the tubes were returned to the water bath.

No label was released from bacteria during treatment with HCl, but after 30 min subsequent treatment with intestinal juice, large amounts of label were released relative to the control. Label continued to be released for up to 3.5 h, but no significant additional release was found thereafter. The total amount of label released was indirectly proportional to pH during treatment with HCl such that the activity released from bacteria treated at pH 1.3 was twice that released from bacteria treated at pH 2.0 (Fig. 1). The amount of label released from bacteria treated at pH 1.3 was similar to that released from bacteria treated with lysozyme. These results show that exposure to low pH lyses bacteria with an efficiency comparable with that of lysozyme and thus makes bacterial cell contents available to digestive enzymes in the intestine.

Gastric pH conditions required for bacterial lyses would be expected to be unfavourable for gastric enzymes. The normal pH of actively digesting vertebrate stomachs is approximately 2.0 and gastric enzymes usually show optimal activity at pH 2 or above. Fish¹⁰ and Nagase¹² report pH optima of 2.2 and 2.8 respectively for protease from the gastric mucosa of *S. mossambicus*, with greatly reduced activity at lower pH. Pooled stomach juices from the ten *S. mossambicus* used in this experiment had a pH of 1.75 and no protease activity was detected. Moriarty⁹ found a similar lack of enzyme activity in the stomach of *Tilapia nilotica* which use gastric acid to lyse blue-green algae. These results suggest that gastric enzymes are not secreted when gastric pH would result in enzyme activities well below optimum.

Of the four important groups of fish which feed on detritus; *Chanos chanos*, mullets, carps and tilapia (including *S. mossambicus*), only tilapia have true stomachs with gastric glands¹⁴. Thus, the mechanism for lysis of detrital bacteria reported here is probably unique to the tilapia, and other mechanisms may await discovery in the other groups.

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STEPHEN H. BOWEN

Institute for Freshwater Studies,
Rhodes University,
Grahamstown, South Africa

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1 Fish, G. R., *Uganda J.*, 19, 85–89 (1955).

- ² Odum, W. E., dissertation, Univ. Miami, cited in Mann, K. H., *Mem. Ist. Ital. Idrobiol.*, **29**, Suppl., 353-383 (1972).
- ³ Mann, K. H., *Mem. Ist. Ital. Idrobiol.*, **29**, Suppl., 13-16 (1972).
- ⁴ Odum, W. E., in *Marine Food Chains* (edit. by Steele, J. H.), 222-240 (University of California Press, 1970).
- ⁵ Newell, R., *Proc. zool. Soc. Lond.*, **144**, 25-45 (1965).
- ⁶ Odum, E. P., and de la Cruz, A. A., in *Estuaries* (edit. by Lauff, G. H.), 383-388 (American Association for the Advancement of Science, Washington DC, 1967).
- ⁷ Heald, E. J., thesis, Univ. Miami, cited in Mann, K. H., *Mem. Ist. Ital. Idrobiol.*, **29** Suppl., 353-383 (1972).
- ⁸ Calow, P., *Hydrobiologia*, **46**, 181-189 (1975).
- ⁹ Wiebe, W. J., and Pomeroy, L. R., *Mem. Ist. Ital. Idrobiol.*, **29**, Suppl., 325-352 (1972).
- ¹⁰ Fish, G. R., *Hydrobiologia*, **15**, 161-178 (1960).
- ¹¹ Moriarty, D. J. W., *J. Zool.*, **171**, 25-39 (1973).
- ¹² Anson, M. L., and Mirsky, A. E., *J. gen. Physiol.*, **22**, 79-89 (1932).
- ¹³ Wright, R. T., and Hobbie, J. E., *Limnol. Oceanogr.*, **10**, 22-28 (1965).
- ¹⁴ Nagase, G., *Z. vergl. Physiol.*, **49**, 270-284 (1964).

Mescaline as a mitotic spindle inhibitor

COLCHICINE and its methyl derivative colcemid not only inhibit microtubule self-assembly¹ but also affect fast transport in nerve². A report that mescaline likewise inhibits the rapid component of axoplasmic transport in nerve³, led us to investigate the effects of this drug on mitosis, and on purified microtubule protein (tubulin⁴). The structure of mescaline does resemble an abbreviated version of colchicine (Fig. 1). We report here that mescaline can, like colcemid, be used as a mitotic spindle inhibitor and has certain advantages as a cytogenetic reagent.

We first investigated the effect of a range of concentrations of mescaline from just above to just below the limits at which concentrations of colcemid exert an inhibitory influence on mitosis. When human skin fibroblasts are cultured in small dishes, the dividing cells round up so that by mid-metaphase they stand out in marked contrast to the flat cells with a polygonal outline characteristic throughout interphase. If the rounded cells are examined using Nomarski interference optics⁵, they can be seen to be in division as one can distinguish the chromosomes in the live cell (Fig. 2). By simply counting the number of rounded cells in a dish, under an ordinary dissecting microscope, one can score any compound for its effect as a mitotic inhibitor.

This first series of experiments (Fig. 3) showed that mescaline was similar to colcemid in its effectiveness. At the higher concentrations, cells treated with colcemid showed some signs of a toxic effect (detachment); those treated with mescaline did not.

A second series of experiments (Fig. 4) designed to study the time course of events up to 24 h after the inoculation of fibroblast cultures with mitotic inhibitors, showed that mescaline is metabolised rather readily, giving a peak of inhibition at 4 h followed by a plateau of moderate inhibition. The first breakdown product of mescaline in human brain is *N*-acetylmescaline⁶. We synthesised this compound,

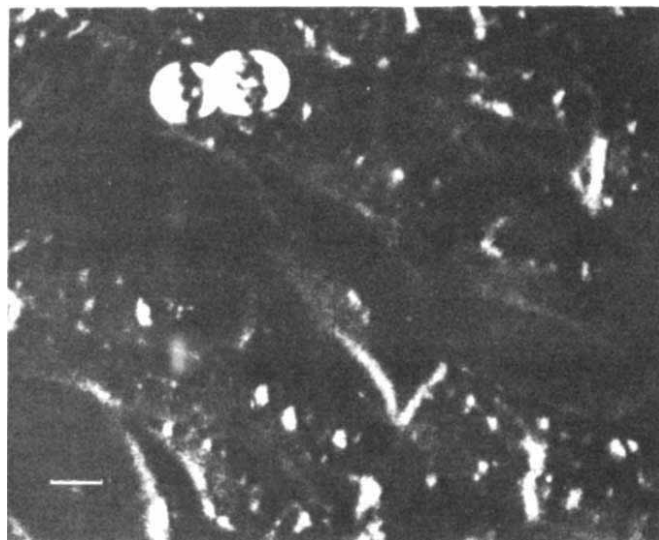


Fig. 2 Nomarski interference image of cells in division. Cells at the top left are in anaphase, with the chromosomes visible on the spindle. The rounded appearance of the dividing cells is in marked contrast with the polygonal interphase cells seen below. The micrograph shows the progress of division past metaphase in the absence of any drug treatment. Bar indicates 20 μ m.

and found that it acts in our experimental system as a moderate inhibitor of mitosis. We therefore attribute this plateau effect to the metabolite *N*-acetylmescaline. Colcemid was also metabolised, but more slowly. It is likely that the first step in this process is the demethylation of a methoxy group in ring A of the molecule, as reported for colchicine in mammalian liver^{7,8}. Results with 1,4-dichlorobenzene, which is known to be an effective inhibitor of mitosis in plants⁹, gave no indication that this agent is metabolised at all.

Our conclusion is that mescaline could be used as a mitotic inhibitor for use in cytogenetic work. Our observations with a variety of human and other mammalian cells show that this drug produces well-spread metaphases suitable for examination by banding techniques (Fig. 5). Mescaline has the advantage that it is 1,000 times less expensive to purchase than is colcemid; it has the disadvantage that its use is controlled by drug legislation.

Our experiments with radioactive mescaline demonstrated that it binds to purified microtubule protein. Like colchicine, it also inhibits the assembly of tubulin subunits to form microtubules. The implications of these findings should be of some general interest. Mescaline is a catecholamine-like neurotransmitter analogue. Its effects on tubulin raise the possibility that neurotransmitters or their

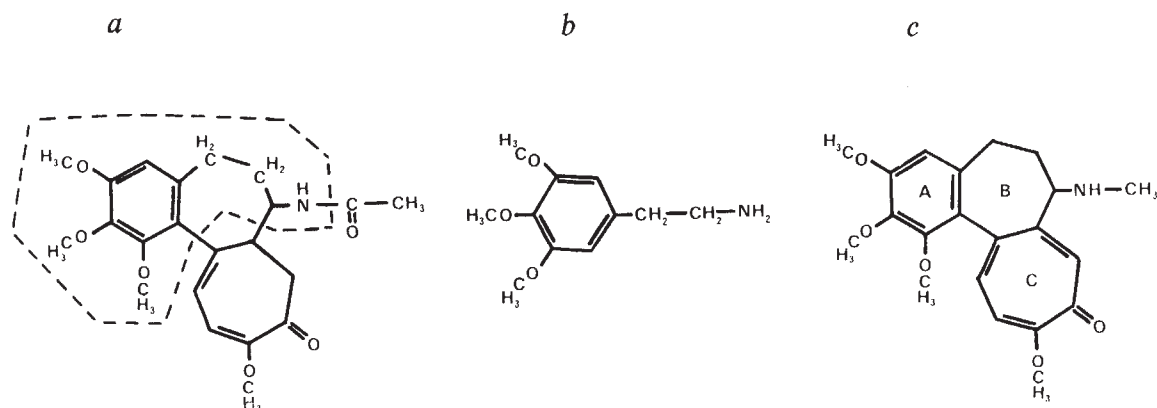


Fig. 1 Structure of spindle inhibitors. *a*, Colchicine; *b*, mescaline; *c*, colcemid. Dotted line indicates the portion of the colchicine molecule to which mescaline would seem to correspond.

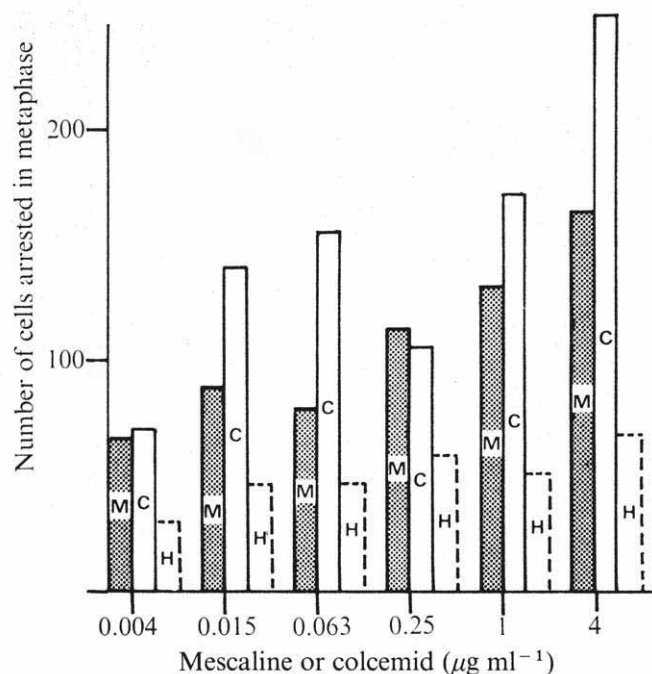


Fig. 3 Comparison of dose responses of human skin fibroblasts to mescaline and colcemid. Cells (2×10^4 per 3-cm Petri dish) were plated out, fed with fresh medium the following day, and exposed to the spindle inhibitors at the dosages indicated. Cell counts were begun 2 h after the start of the treatment and are expressed as the average of a forward and a backward count through the series of dishes. Slight effects of cooling and disturbance are reflected by minor increases in counts in the control dishes. Cells rounding up due to toxic effects at high dosage of colcemid may make some contribution to the count at $4 \mu\text{g ml}^{-1}$. M, Dishes given mescaline in 0.1 ml Hanks' balanced saline; C, dishes given colcemid in 0.1 ml Hanks' balanced saline; H, control dishes given 0.1 ml Hanks' balanced saline.

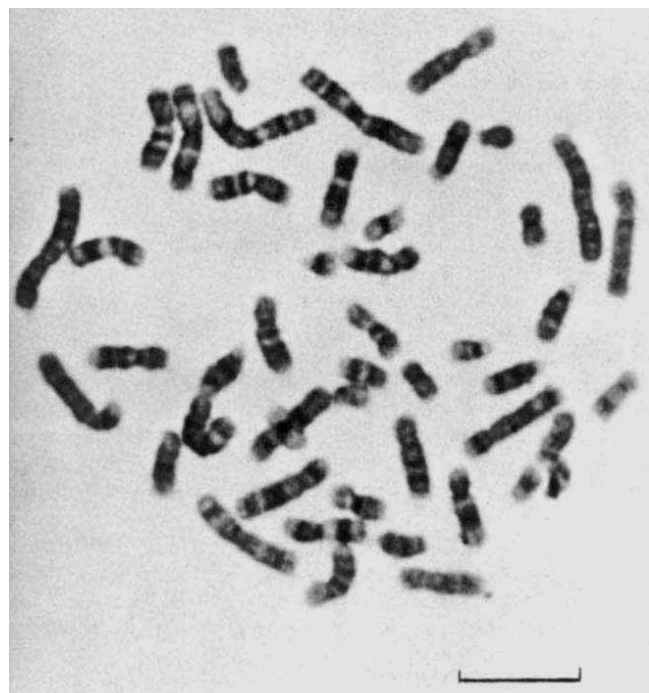


Fig. 5 Metaphase spread from a human leukocyte culture prepared with mescaline as a spindle inhibitor, trypsin-banded and stained with Giemsa. Bar indicates $10 \mu\text{m}$.

metabolites may have a separate role additional to their effect at the cell membrane—regulation of the polymerisation state of fibrous proteins within the cell. The biochemical results of this work will be reported separately.

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C. M. H. HARRISSON
B. M. PAGE
H. M. KEIR

Departments of Biochemistry and Genetics,
University of Aberdeen,
Aberdeen AB9 1AS, UK

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- 1 Weisenberg, R. C., *Science*, **177**, 1104–1105 (1972).
- 2 Marchisio, P. C., Aglietta, M., and Rigamonti, D., *Experientia*, **29**, 1126–1127 (1973).
- 3 Paulson, J. C., and McClure, W. O., *Molec. Pharmacol.*, **9**, 41–50 (1973).
- 4 Mohri, H., *Nature*, **217**, 1053–1054 (1968).
- 5 Nomarski, M. G., *J. Phys. Radium, Paris*, **16**, 9S–16S (1955).
- 6 Charalampous, K. D., Walker, K. E., and Kinross-Wright, J., *Psychopharmacologia*, **9**, 48–63 (1966).
- 7 Schönharting, M., Mende, G., and Siebert, G., *Hoppe Seyler's Z. physiol. Chem.*, **354**, 1242–1243 (1973).
- 8 Schönharting, M., Pfänder, P., Rieker, A., and Siebert, G., *Hoppe Seyler's Z. physiol. Chem.*, **354**, 421–436 (1973).
- 9 Gavaudan, P., Gavaudan, M., and Durand, J. -F., *C. r. hebdomadaire Seances Acad. Sci., Paris*, **208**, 593–595 (1939).

Antibodies to a cell-surface component coded by human chromosome 21 inhibit action of interferon

ANTICELLULAR sera able to block specific cell responses are important in the study of surface receptors. Particularly useful are antibodies against the human cellular antigens coded for by a specific chromosome, which can be prepared by immunising mice with mouse-human somatic cell hybrids containing defined human chromosomes¹. We have applied this method to the study of the mechanism by which the presence of human chromosome 21 renders such hybrids sensitive to human interferon². Interaction of an interferon with sensitive cells induces intracellular biochemical changes, including the formation of an inhibitor of protein synthesis, making the cell unable

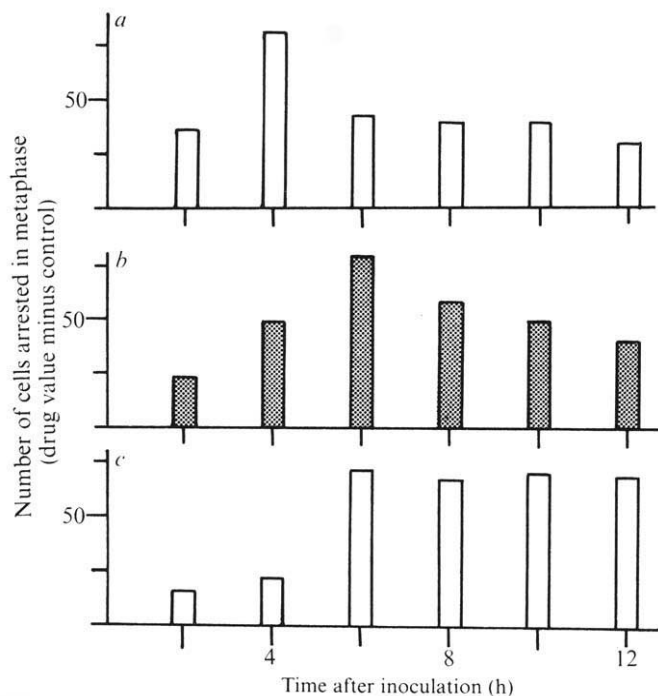


Fig. 4 Time course of effects of spindle inhibitors on human skin fibroblasts. Cells were plated out as in Fig. 3. Counts were made once on each of six sets of dishes at 2 h intervals, each set included a control inoculated with Hank's balanced saline, a dish with colcemid (a) at $4 \mu\text{g ml}^{-1}$, a dish with mescaline (b) at $5 \mu\text{g ml}^{-1}$, and a dish inoculated with 0.1 ml saturated solution (c) of 1,4-dichlorobenzene in Hanks' balanced saline.

to support virus replication^{3,4}. We have obtained antibodies to hybrid cells containing human chromosome 21, and these inhibit the response of live human cells to interferon. Our experiments indicate that chromosome 21 codes for a cell-surface component required as a specific receptor for human interferon.

For immunisation, C3H/FeJ mice were inoculated intraperitoneally once a week with 10^7 cells from clone WAVR4d (ref. 5) originating from the fusion of a mouse A9 cell and a human diploid fibroblast WI-38: this clone contains human chromosomes 4, 21 and 22 only. After 4 weeks, immune serum was collected and decomplexed at 56 °C for 30 min. The titre of antibodies was estimated by cytotoxicity tests⁶ in the presence of rabbit complement. At a dilution of 1/128 the killing index was 70% on WAVR4d and 20% on mouse A9 cells. At 1/32, they were 90 and 70, respectively. The response to mouse cell was lowest for short periods of immunisation. Adsorption of the antiserum on A9 cells eliminated the mouse-specific reaction, while keeping intact the reaction against hybrid cells. No reaction was seen with hybrids whose human chromosomes do not include 4, 21 or 22. Reaction was observed with HeLa cells. Most important, no cytotoxicity was observed in the absence of complement, which is the condition used in the interferon assay. Human interferon (5×10^4 U ml⁻¹) was prepared in foreskin cells FS4 (given by Dr J. Vilcek) by induction with poly(rI)-poly(rC)⁷.

Effects of anti-WAVR4d serum were first studied on the line of hybrid cells used for immunisation (Table 1). These cells contain human chromosome 21 and are therefore sensitive to human interferon (although they require at least 10 times more interferon than normal human cells). Hybrids lacking chromosome 21 do not respond to human interferon. Addition of the antiserum completely inhibits the effects of human interferon on vesicular stomatitis virus (VSV) replication in the sensitive hybrid cells. To establish that the inhibition of interferon's action is due to reaction with human immunodeterminants, we next examined the effect of the antihybrid serum on normal human diploid fibroblasts.

Pretreatment of human foreskin cells FS7, with anti-WAVR4d serum inhibited the antiviral effect of interferon (Fig. 1). At low interferon concentrations, the inhibition of VSV replication is completely abolished after exposure to the antibodies. Excess of interferon can overcome the effects of antiserum pretreatment. At intermediate interferon concentrations, more than 100 times more virus is produced in the cells exposed to the antiserum. The antiserum against hybrid cells containing only few human chromosomes is therefore active also against human diploid cells. Several independent immunisation experiments gave the same results.

The specificity of the anti-interferon effect was investigated after exhaustive adsorption of the antiserum on hybrid cells from lines either 4+21-22+ or 4-21+22-. Table 2 shows that

Table 1 Effect of antiserum on action of interferon in hybrid cells

Additions	VSV yield in mouse-human hybrids	
	WAVR4d (21 ⁺)	JFA16a (21 ⁻)
	(log PFU ml ⁻¹)	
None	7.07	6.23
Interferon, 50 U	4.53	7.08
Anti-WAVR4d serum, 1:64	7.92	—
Antiserum + interferon	7.07	—

Interferon assays were carried out in microplates II (96 wells, Falcon) containing 2.5×10^4 cells per well in 0.1 ml Dulbecco's modified Eagle's medium—10% foetal calf serum (DVME). Incubations were at 37 °C. Antiserum adsorbed on mouse A9 cells (10^7 per ml serum) was added as indicated, followed after 1 h by human interferon. After 7 h, cells were washed and infected with 2.5×10^5 PFU VSV in Eagle's MEM—2% FCS. After 24 h, medium in each well was collected and the VSV titre was determined by the micromethod of Rosenthal and Shechmeister¹⁴. VSV titres are expressed in log PFU ml⁻¹. Karyotypes of the hybrid cells used were WAVR4d: 4,21,22; JFA 16a: 2,5,11,16,17,18 and 20.

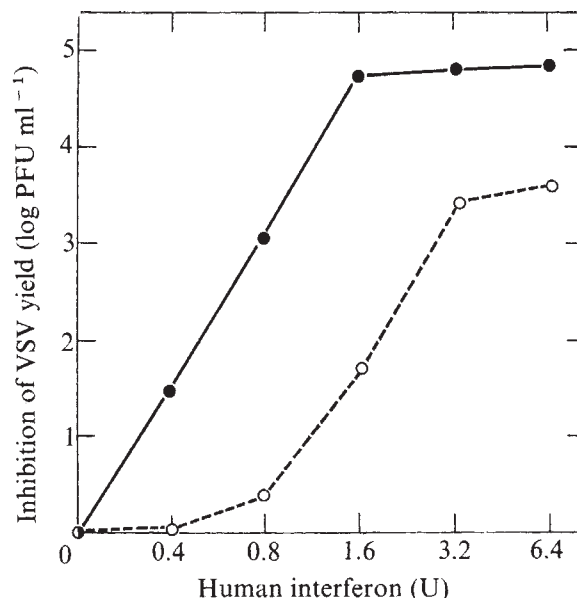


Fig. 1 Inhibition of the interferon effect by antihybrid serum applied to normal human diploid foreskin cells, FS7 (given by Dr J. Vilcek). Assay as in Table 1: to wells containing 2.5×10^4 cells in DVME—10% FCS, anti-WAVR4d serum (adsorbed on 10^7 mouse A9 cells per ml serum) was added at a dilution of 1/128. One hour later, the indicated amounts of interferon were added. After 7 h, cells were washed and VSV growth was measured as in Table 1. The virus yield in log PFU ml⁻¹, without interferon, was 7.28. The reduction in log PFU ml⁻¹ VSV, caused by interferon, is plotted. ●, Control; ○, antiserum.

the anti-interferon activity of the antiserum is lost only after adsorption on cells containing human chromosome 21. Furthermore, an antiserum prepared against mouse-human hybrid cells containing chromosome 6, 8 and X (but not 21) does not inhibit the antiviral effect of interferon even at high concentrations (experiment 2, Table 2).

These data strongly implicate chromosome 21 as determining the antigen responsible for the serum's anti-interferon effect. We therefore compared the response of human fibroblasts either monosomic or trisomic for chromosome 21. The results

Table 2 Specificity of anti-interferon effect of antiserum for chromosome 21

Antiserum used	VSV yield	
	Inhibition by interferon (log PFU ml ⁻¹)	Antiserum effect
Experiment 1		
(1) No antiserum	3.6	—
(2) With anti-WAVR4d, 1:64		
—adsorbed on mouse A9 cells	1.7	1.9
(3) —readsorbed on hybrid 4+21-22+ cells	1.7	1.9
(4) —readsorbed on hybrid 4-21+22- cells	3.4	0.2
Experiment 2		
(5) No antiserum	1.9	—
(6) With anti-Hal-6 serum, 1:16	2.1	0.0
(7) With anti-WAVR4d serum, 1:16	0.0	1.9

Experiment 1: anti-WAVR4d serum was first adsorbed by incubation for 30 min at 25 °C with 10^7 mouse A9 cells per ml of serum diluted 1/32 in protein-free DVME. After centrifuging out the cells, antiserum was either used directly (line 2) or readsorbed at 2×10^7 cells per ml on mouse-human hybrid AIM3a (2,3,4,5,7,10,12,13,14,17,19,22,X) (line 3) or AHA-16c (3,11,13,21,X) (line 4). Assay as in Fig. 1 with 1.6 U human interferon in 0.1 ml containing 2.5×10^4 human foreskin FS7 cells. Yield of VSV was 7.92 log PFU ml⁻¹ without interferon. For experiment 2, mice were immunised with either mouse-human hybrids HAL (6,8,X) or WAVR4d (4,21,22) and the antisera obtained assayed as above. Yield of VSV was 7.08 log PFU ml⁻¹ without interferon.

showed that the effect of the antiserum is stronger in monosomic 21 cells and decreases with increasing number of chromosomes 21 in the target cells. Because to produce the same inhibition in VSV replication, the monosomic line had to receive five times more interferon than the trisomic line⁸ (Fig. 2), the antiserum effect is clearly determined by the cell and not by the interferon. Together with the fact that the hybrid cells WAVR4d, which served as immunogen, cannot produce human interferon (since they lack chromosomes 2 and 5) (ref. 9), these results enable us to conclude that the anti-interferon effect is due to antibodies directed against a cellular component different from interferon itself. This component, coded for by some gene(s) on chromosome 21, is required for the action of interferon.

Several observations indicate that the antiserum inhibits the action of interferon by combining with a component of the cell surface: (1) the effect is seen with living cells in which only the surface antigens are available for reaction with the antibodies¹⁰; (2) the inhibition is reversible: excess of interferon overcomes the inhibition caused by pretreatment of the cells with antibodies (Fig. 1); (3) immunofluorescence studies showed that the antiserum used in this work stains the surface of live human fibroblasts. We propose that this cell-surface component coded for by chromosome 21, serves as a receptor for human interferon. This interpretation is supported by the comparison of cells with different doses of chromosome 21. Figure 2 shows that chromosome 21 controls the amount of interferon needed to reach a given level of cell response, but not the final antiviral state achieved with saturating concentrations of interferon. This is expected if some gene on this chromosome codes for a receptor to human interferon; if, on the other hand, it would code for an intracellular antiviral protein (AVP) as suggested⁸, the final antiviral state should be higher in trisomic than in

monosomic 21 cells. In the mouse-human hybrids, the location of the gene for such an antiviral protein, made in response to interferon, remains undetermined; one possibility is that it needs not to be specifically human but may be a mouse gene.

Human interferon binds rapidly to human cells¹¹. Whether this first binding, which may involve gangliosides¹², triggers the antiviral state is still debatable¹³. We have found recently that even after 3 h of contact at 37 °C between interferon and human fibroblasts, the establishment of the antiviral state is still blocked by our antiserum. This indicates that a prolonged interaction of interferon with cell-surface receptors is necessary to induce the antiviral state. Our antiserum should prove useful for further study of cell-interferon interactions.

More generally, antisera against cell-surface determinants coded by given human chromosomes may select in a hybrid population against cells containing these determinants, and provide a selection method for the loss of specific human chromosomes.

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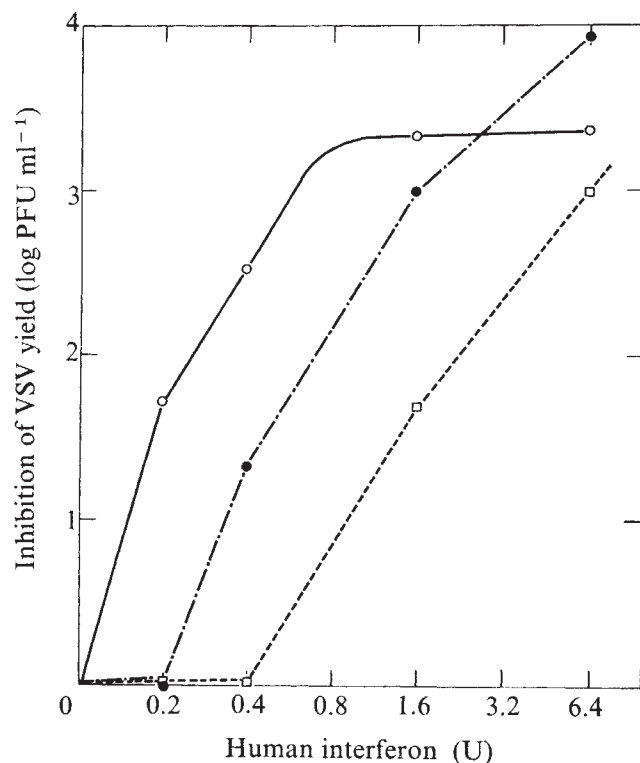
M. REVEL
D. BASH
F. H. RUDDLE

Department of Biology,
Yale University, New Haven,
Connecticut 06520 and
Virology Section,
Weizmann Institute of Science,
Rehovot, Israel.

Received November 13, 1975; accepted January 26, 1976.

- 1 Buck, D. W., and Bodmer, W. F., in *Human Gene Mapping*, 6, 87-89 (Kaizer, Basel, 1975).
- 2 Tan, Y. H., Tishfield, J. A., and Ruddle, F. H., *J. exp. Med.*, **137**, 317-330 (1973).
- 3 De Clercq, E., and Stewart, W. E., II, in *Selective Inhibitors of Viral Functions* (edit. by Carter, W. A.), 81-107 (Chemical Rubber Co., Cleveland, 1973).
- 4 Revel, M., et al., in *Perspectives in Virology*, 9, (edit. by Pollard, M.), 233-248 (Academic, New York, 1975).
- 5 Tishfield, J. A., and Ruddle, F. H., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 45-49 (1974).
- 6 Bash, J. A., Dubiski, S., and Cinader, B., *Int. Archs Allerg.*, **47**, 369-379 (1974).
- 7 Havell, E. A., and Vilcek, J., in *The Production and Use of Interferon for the Treatment and Prevention of Human Virus Infections*, *In Vitro* monogr. No. 3, 47, (Tissue Culture Association, 1974).
- 8 Tan, Y. H., *Nature*, **253**, 280-282 (1975).
- 9 Tan, Y. H., Creagan, R. P., and Ruddle, F. H., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2251-2255 (1974).
- 10 Nicolson, G. L., in *Membrane Research* (edit. by Fox, C. F.), 53-70 (Academic, New York, 1972).
- 11 Bermann, B., and Vilcek, J., *Virology*, **57**, 378-386 (1974).
- 12 Besancon, F., and Ankel, H., *Nature*, **252**, 478-480 (1974).
- 13 Kohno, S., Buckler, C. E., Levy, H. B., and Baron, S., *IRCS Medical Sciences*, **3**, 57 (1975).
- 14 Rosenthal, L. J., and Shechmeister, I. L., in *Tissue Culture Methods and Applications*, (edit. by Kruse, P. F., and Patterson, M. K.), 509-516 (Academic, New York, 1973).

Fig. 2 Comparison of human fibroblasts monosomic, disomic and trisomic for chromosome 21, in their response to human interferon. Lines GM 258 (trisomy 21) and GM 230 (monosomy 21) were obtained from the National Institute of General Medical Sciences genetic mutant cell repository (Camden). The diploid line was foreskin cells FS4. Interferon assay as in Fig. 1, except that treatment was for 24 h before VSV infection. The virus yield, after 29 h was in log PFU ml⁻¹ 6.67 for FS4 and 5.38 for GM 258 and GM 230. ○, Trisomy 21; ●, diploid; □, monosomy 21.



Chromosome 21 and the cell growth inhibitory effect of human interferon preparations

INTERFERONS constitute a class of biologically active glycoproteins¹⁻³ which induce a spectrum of physiological changes in living cells, leading to the inhibition of virus replication⁴, of cell growth *in vitro*—both normal and tumour cells^{5,8}—and of tumour growth *in vivo*⁹⁻¹⁵. The mechanism(s) underlying these effects is unknown. Gressor *et al.* have suggested that the same molecule in an interferon preparation is responsible for both the antiviral (AV) and cell growth inhibitory (CGI) effect³. This suggestion was based on the observation of a consistent and direct correlation between the two effects in an interferon preparation purified more than a millionfold. Gressor also reported that tumour cells treated with interferon had a

lower capacity to form colonies in Agarose and were less tumorigenic when inoculated¹⁵, suggesting that the antitumour effect of interferon depended, in part, on the inhibition of tumour cell multiplication which can be demonstrated *in vivo*. I describe here a genetic approach to ascertain whether the CGI effect of human interferon preparations is mediated by chromosome 21-directed gene(s), known to govern the AV action of interferon¹⁶⁻¹⁹. Such an approach, should provide further insight into whether the different effects of interferon are governed by a cluster of genes on chromosome 21 or whether a single genetic locus on chromosome 21 codes for the pleiotropic effects of interferon.

The CGI and AV effects of two human interferon (HIF) preparations were assayed on primary human skin fibroblasts containing different copies of chromosome 21. These cells are monosomic (M21), disomic (D21) and trisomic (T21) for chromosome 21. Primary human cells carrying chromosomal translocations were also used: (1) a diploid cell line (t4;21), in which one of the two chromosomes 21 is translocated to the short arm of chromosome 4, with the resultant loss of the proximal short arm of chromosome 21 (ref. 20); (2) a trisomic 21 cell line (t6;21), in which one of its three chromosomes 21 is translocated to chromosome 6, with the resultant loss of the centromere as well as the short arm of chromosome 21 (ref. 21), (3) another diploid cell line (t4;2), carrying a 2.4 chromosomal translocation, and (4) a trisomic 21 cell line (T21;t-17), carrying a 1-17 chromosomal translocation²². A primary human skin fibroblast trisomic for chromosome 18 is also used. The CGI and AV effects of interferon were assessed in these cells by assaying the amount of interferon required to inhibit the *in vitro* growth of cells and synthesis of vesicular stomatitis virus, respectively, by 50%. Human cells which were less sensitive to the CGI effects of interferon were also less sensitive to its AV action (Table 1) confirming a similar observation in L1210 cells with differential sensitivity to mouse interferon²³. Table 1 also shows that the sensitivity of human cells to both the CGI and AV actions of interferon varied inversely with the number of copies of human chromosome 21 present in the cell. Similarly, Fig. 1 shows that M21 cells were less sensitive than D21 cells, which in turn were less sensitive than T21

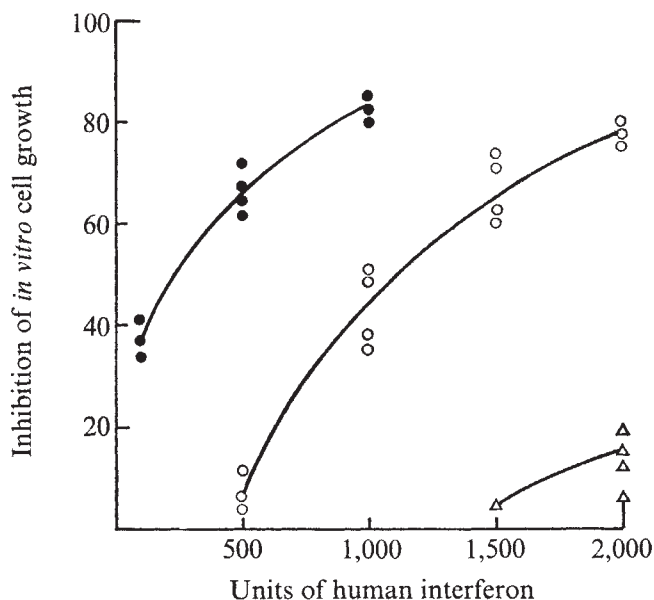


Fig. 1 Effect of human leukocyte interferon on the *in vitro* cell growth of trisomic 21 (●), disomic 21 (○) and monosomic 21 (△) human skin fibroblasts. Each point on the graph represents the results from one determination.

cells to the CGI effect of interferon. It is unlikely that these observations were due to a nonspecific increase in the number of human chromosomes because the T18 cells, which contain an extra copy of chromosome 18 but have the normal complement of chromosomes 21, behave like a D21 cell in terms of sensitivity to the CGI effect. These results suggest that chromosome 21 contains the gene(s) governing both the CGI effect and the AV action of interferon. Table 1 also shows a dose effect of translocated chromosome 21 on CGI activity. This translocated dose effect has been used to assign the gene(s) governing the AV action of interferon to the long arm of chromosome 21 (ref. 24). The translocated 21 chromosomes (the long arm portions) in both the t4;21 and t6;21 lines exert a dose effect on these cells such that t4;21 and t6;21 cells were as

Table 1 Response of cells to interferon

Cell type	Identification no.	NIH-1F	Cell growth	Units of HIF inhibiting by 50%	
				108-1F	Viral RNA synthesis NIH-1F
M21	230	> 2,500	> 2,500	> 2,500	1.8
	198	> 2,500	> 2,500	> 2,500	1.7
D21	141	1,664, 1,860	800, 1,100	0.42	0.48
	115	1,760, 1,902	825, 985	0.48	0.50
	15	1,458, 1,264	924, 1,024	ND	ND
T21	258	256, 240	40, 80, 95	0.11	0.10
	114	212, 236	64, 112, 118	0.12	0.14
	14	224, 301	112, 116, 142	ND	0.09
T18	143	1,560	860, 1,114	0.50	ND
t4; 2	301	1,811, 1,763	1,030, 1,004	0.44	0.45
t4; 21	98	1,988, 2,010	1,306, 1,114	0.41	0.40
t6; 21	144	255, 276	48, 36, 72	0.08	0.09
t21;t-17	201	199, 288	42, 134, 104	0.09	0.12

NIH-1F, Sendai-induced human leukocyte interferon reference reagent G023-901-S-27.

108-1F, poly(I)-poly(C)-induced human fibroblastoid interferon from a high producing cell line V-M15-clone 108.

For the cell growth inhibitory assay, 2×10^4 cells were seeded per 25 cm² flask and maintained in 5 ml of regular growth medium (MEM medium containing 10% of heat-inactivated foetal bovine serum, 2 mM glutamine and 1% chlorotetracycline) for 20-24 h. The test flasks were reincubated with different concentrations of human interferon in 5 ml of regular medium. The cultures were incubated at 37 °C for 6 d. On day 6 cultures were trypsinised and total cells were counted with a Coulter counter. The CGI effect was assessed by the amount of HIF required to inhibit the total cell count by 50%. Control cultures were treated the same as the test cultures, except that interferon was omitted. Each figure represents average results obtained from one set of duplicate cultures.

sensitive as D21 and T21 cells, respectively, to the CGI activity of interferon (Table 1). It is unlikely that the effects observed with these translocations were due to a nonspecific chromosomal translocation, because two other translocations—the 4-2 translocation in the t4;2 line containing two copies of chromosome 21 and the 1-17 translocation in the T21 cells (T21;1-17) line containing three copies of chromosome 21—behaved like D21 and T21 cells, respectively, in terms of their sensitivity to the CGI effect. The results suggest that the CGI effect like the AV action of interferon is governed by gene(s) located on the long arm of chromosome 21.

Table 1 shows that for each unit of AV activity, human fibroblastoid interferon (108-1F) was more inhibitory to growth than human leukocyte interferon (NIH-1F). This is not surprising since the two interferons are biologically and antigenically different²⁵⁻²⁷. But whether this means that human fibroblastoid interferon is potentially more effective than leukocyte interferon as an antitumour agent is subject to further study.

Of the two interferons used in this study, 108-1F was a poly(I)·poly(C)-induced interferon from our high producing line of human aneuploid cells containing multiple copies of the chromosomes associated with interferon production^{19,28}. To eliminate the possibility that the CGI effect observed with this preparation was due in part to residual poly(I)·poly(C), cells were grown in medium containing the concentrations of poly(I)·poly(C) indicated in Table 2. There was no inhibition of cell growth at these concentrations of poly(I)·poly(C), rather, at the lower concentrations, a small stimulation of cell growth was apparent.

Table 2 Effect of poly(I)·poly(C) on growth of primary human skin fibroblast cultures

poly(I)·poly(C) ($\mu\text{g ml}^{-1}$)	Total cell count of cultures	
	Treated	Not treated
0.09	1.1×10^5	1.3×10^5
0.05	1.3×10^5	0.9×10^5
0.009	1.4×10^5	1.1×10^5
0.001	1.2×10^5	0.8×10^5

Human cells trisomic for chromosome 21 (line 114) were seeded at 2×10^4 cells per 25 cm^2 flask and maintained in regular growth medium for 20–24 h. The medium was removed from each flask and reincubated with growth medium containing the above concentrations of poly(I)·poly(C) (P.L. Biochemicals) for 6 d. Poly(I)·poly(C) was omitted from controls. Each figure represents average counts from one set of duplicate cultures.

To interpret the significance of these findings, a model is presented to relate the role of chromosome 21 to the pleiotropic effect of interferon. According to this model, a single gene or a cluster of genes located on the long arm of chromosome 21, govern(s) the response of human cells to interferon. One of the first events after the treatment of cells with interferon is the interaction between interferon, or an inhibitory growth substance which has been coinduced and/or coreleased with interferon, with their respective receptor sites. These sites could also be specified by the chromosome 21-directed genes. (There is no direct evidence for the existence of these receptor sites, although they have been inferred from studies with interferon bound on Sepharose beads²⁹.) Then the chromosome 21 gene(s) is stimulated, presumably by the events at the cell surface^{18,19,30}, to transcribe gene product(s) which in turn are directly responsible for mediating the pleiotropic effects of interferon. Evidently, a newly induced second protein is implied here. But unlike the induction of the AV state, there is no evidence that a second protein is required for the CGI effect. For this reason an alternative explanation is offered. Presumably, again, interferon or the inhibitory growth factor interacts with a chromosome 21-directed gene product(s). This interaction produces chemical and/or conformational change(s) which interfere(s), with the replication of viruses and the

normal rates of cell division. For this alternative, it is necessary to postulate that these gene product(s) are synthesised constantly and turned over rapidly. This assumption then explains the inhibition of the AV effect by actinomycin D (ref. 31) as well as the lack of the AV induction in enucleated cells³². Another alternative is that an interaction between interferon or inhibitory growth factor and chromosome 21 gene(s)-directed cell-surface component(s) is sufficient to cause cell-surface changes. These in turn inhibit the normal rates of cell division but not viral replication, which requires a second protein, the AV protein. To decide between these alternatives, it is essential to identify and isolate the gene product(s) in question. Until then, it would be worthwhile to consider these alternatives equally.

A natural sequel to this is to apply a similar approach to ascertain whether chromosome 21 also controls the anti-tumour effect of human interferon. As might be anticipated from the suggestion that the anti-tumour effect depends in part on the CGI effect, it will be feasible to design *in vitro* tests using this effect to predict the human tumour types that will be most responsive to interferon therapy.

Y. H. TAN*

Division of Medical Biochemistry,
Faculty of Medicine,
University of Calgary,
Calgary, Alberta T2N 1N4, Canada

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* Present address: Visiting Investigator, International Laboratory Molecular Biology Interferon System, Arnold Schwarz Hall Cancer Research, Sloan-Kettering Research Center, New York 10021.

- 1 Knight, E., *J. biol. Chem.*, **250**, 4139–4144 (1975).
- 2 Dorner, F., Scriba, M., and Weil, R., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1981–1985 (1973).
- 3 Besancon, F., and Bourgeade, M., *J. Immun.*, **113**, 1061–1063 (1974).
- 4 Issacs, A., and Lindemann, J., *Proc. R. Soc. B*, **147**, 258–267 (1957).
- 5 Gressor, I., et al., *Proc. Soc. exp. Biol. Med.*, **142**, 7–10 (1973).
- 6 Macieria-Coelho, A., Brouty-Boye, D., Thomas, M. T., and Gressor, I., *J. Cell Biol.*, **48**, 415–419 (1971).
- 7 Paucker, K., Cantell, K., and Henle, W., *Virology*, **17**, 324–334 (1962).
- 8 Adams, A., Strander, H., and Cantell, K., *J. gen. Virol.*, **28**, 207–217 (1975).
- 9 Salerno, R. A., Whitmire, C. E., Garcia, I. M., and Heubner, R. J., *Nature new Biol.*, **239**, 31–32 (1972).
- 10 Gressor, I., Thomas, M. T., and Brouty-Boye, E., *Nature*, **231**, 20–21 (1971).
- 11 Kassel, R. L., *Clin. Obstet. Gynec.*, **13**, 910–927 (1970).
- 12 Came, P. E., and Moore, D. H., *Proc. Soc. exp. Biol. Med.*, **137**, 304–305 (1971).
- 13 Gressor, I., Maury, C., and Brouty-Boye, D., *Nature*, **239**, 167–168 (1972).
- 14 Strander, H., and Cantell, K., *Int. Workshop in Interferon in the Treatment of Cancer* (Sloan-Kettering, New York, 1975).
- 15 Gressor, I., *Int. Workshop in Interferon in the Treatment of Cancer* (Sloan-Kettering, New York, 1975).
- 16 Tan, Y. H., Tischfield, J., and Ruddle, F. H., *J. exp. Med.*, **137**, 317–330 (1973).
- 17 Tan, Y. H., Schneider, E. L., Tischfield, J., Epstein, C., and Ruddle, F. H., *Science*, **186**, 61–63 (1974).
- 18 Tan, Y. H., *Nature*, **253**, 280–282 (1975).
- 19 Tan, Y. H., *Effects of Interferon on Cells, Viruses and the Immune System* (edit. by Gerald, A.), 3–31 (Academic, New York, 1975).
- 20 Seabright, M., Miller, R. C., Greene, A. E., and Coriell, L. L., *Cytogenet. Cell. Genet.*, **14**, 152–153 (1975).
- 21 Borgaonkar, D. S., Greene, A. E., and Coriell, L. L., *Cytogenet. Cell. Genet.*, **13**, 403–405 (1974).
- 22 Chapelle, A., de la Miller, R. C., Greene, A. E., and Coriell, L. L., *Cytogenet. Cell Genet.*, **14**, 82–83 (1975).
- 23 Gressor, I., Thomas, M. T., and Brouty-Boye, D., *J. natn. Cancer Inst.*, **52**, 553–559 (1970).
- 24 Tan, Y. H., and Greene, A. E., *J. gen. Virol.* (in the press).
- 25 Anfinsen, C., Base, S., Corley, L., and Gurari-Rotman, D., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3139–3142 (1974).
- 26 Stewart, W. E., II, *Int. Workshop Interferon in the Treatment of Cancer* (Sloan-Kettering, New York, 1975).
- 27 Havell, E., et al., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2185–2187 (1975).
- 28 Tan, Y. H., *Symp. Antiviral with Clinical Potentials*, A-1 Stanford University, Palo Alto (1975).
- 29 Ankel, H., Chany, C., Galliot, B., Chevalier, M. J., and Robert, M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2360–2363 (1973).
- 30 Chany, C., et al., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 3129–3133 (1975).
- 31 Taylor, J., *Biochem. biophys. Res. Commun.*, **14**, 447–448 (1964).
- 32 Kates, J. R., Radke, K. L., and Colby, C., *Abstr. A. Meeting, Am. Soc. Microbiol.*, **V267** (1975).

Spermatogenic failure of translocation heterozygotes affected by H-2-linked gene in mouse

ACCORDING to Lifschytz and Lindsley^{1,2}, the basic control mechanism of spermatogenesis of all male heterogametic organisms consists of inactivation of the X chromosome during differentiation of germ cells. The absence of this

control mechanism in female meiosis, where both X chromosomes are active^{3,4}, could explain why only male gametogenesis is impaired in the carriers of some chromosomal aberrations.

Here, the first data are reported on a genetic factor located on the 17th murine chromosome which controls the range of spermatogenic impairment of mice heterozygous for T(14;15)6Ca translocation¹⁰ (abbreviated, T6).

I have previously envisaged⁵ a possible mechanism which would relate the male sterility of autosomal translocation carriers to the impairment of X-chromosome inactivation.

The hypothesis of Lifschytz and Lindsley^{1,2} fits well with the data on spermatogenic breakdown caused by X-autosomal translocations and certain X-chromosome deficiencies^{1,2} but it apparently could not account for sterility caused by many autosomal reciprocal translocations⁶⁻⁸. Nevertheless, several characteristic features of both types of translocation indicate a possible common mechanism of male infertility: (1) the sterility is clearly chromosomal in both types of translocation heterozygotes; (2) only male carriers are sterile; and (3) infertility is caused by the breakdown of spermatogenesis.

My idea⁵, outlined above and based on the observation of a non-random tight association of the X chromosome with translocation multivalent in primary spermatocytes, has been supported by an analogous cytological picture found in one tertiary trisomic male mouse⁹ and in the other male-sterile translocation (J.F., unpublished). These data, however, give little information on the causal relationship between the X-autosomal attachment in meiosis and the subsequent spermatogenic failure. Thus, another approach to the analysis of sterility of autosomal translocation carriers, might seem desirable.

The genic component of variation in the severity of spermatogenic failure of males heterozygous for T6 translocation is revealed when the particular types of F₁ hybrids from crosses of CBA-T6/T6 males with females of nine inbred strains are compared (Table 1). The symbol CBA-T6/T6 designates a homozygous T6 translocation carried on the genetic background of CBA inbred strain. By comparing the distributions of testes weight values, which are sensitive measures of spermatogenic impairment¹¹⁻¹³, the three phenotype classes of testes weight values (high, medium and low) are distinguished among the nine types of F₁ hybrids. The mean number of spermatozoa in epididymides, which is another criterion of spermatogenic failure¹⁴, shows reasonable correlation with testes weight in spite of variations in individual scores (Fig. 1).

Table 1 shows further that spermatogenic failure dis-

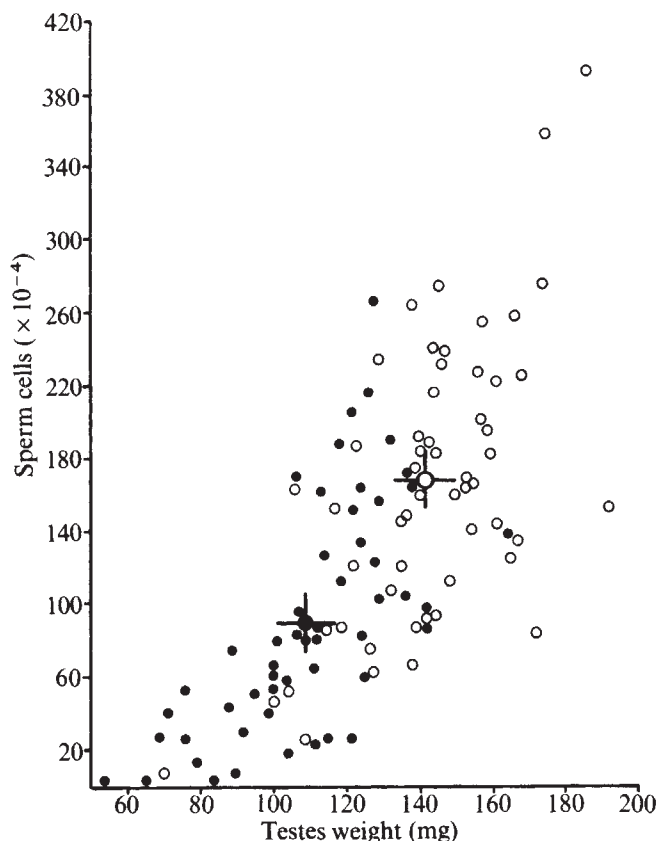


Fig. 1 Number of spermatozoa in paired epididymides plotted against testes weight in T6 heterozygous male progeny of CBA-T6/T6 × (C57BL/10-T × C3H)/+ cross. The tested males, 60 ± 4 d-old, were genotyped as *H-2^b/H-2^k* (●) or *H-2^k/H-2^k* (○) by PVP haemagglutination²¹ with specific anti-*H-2^b* sera. The mean values of *H-2^b/H-2^k* males (crossed ●), (109 mg, 90 × 10⁴ sperm cells) and *H-2^k/H-2^k* males (crossed ○), (141 mg, 164 × 10⁴ sperm cells) differ significantly (Hotellings *T*² statistics = 31.762; *P* < 0.01) which indicates that a gene linked to *H-2^b* on chromosome 17 from C57BL/10-T strain is the major genetic determinant responsible for low testes weights and low sperm counts. Fourteen recombinants between *T* and *H-2* were detected among 107 males tested; however, they do not provide sufficient information for location of the postulated gene with respect to *T* and *H-2* loci, partly because of a physiological component in variation of fertility parameters, and partly because of difficulties with classification of some *T*/+ compared with +/+ animals on this particular genetic background. Therefore, for the sake of clarity, only the *H-2* genotype is shown.

Table 1 Testes weights and sperm counts in T6 heterozygous male progeny of (inbred strain × CBA-T6/T6) cross

Inbred strain*	Mean testes weight† (mg)	s.e.	Sample size‡ T/S	Number of spermatozoa§ × 10 ⁻⁴
C3H/Di	154 (H)	2.3	13/7	158
A/Ph	120 (M)	2.5	14/14	120
BALB/c	119 (M)	1.8	13/6	133
F/St	119 (M)	2.2	8/0	ND
I/St	125 (M)	2.1	20/9	178
AKR/J	99 (L)	3.9	12/12	97
C57BL/10	98 (L)	1.1	54/27	49
C57BL/6	98 (L)	2.7	19/7	16
CBA/J	93 (L)	3.6	11/8	40
Homozygous (T6/T6 and +/+) control males				
CBA-T6/T6	138	2.0	16/14	221
CBA/J	143	4.5	16/16	245

*Female parent.

†Wet weight of paired testes of males aged 60 ± 4 d; H, M, L, high, medium and low testes weight.

‡Number of F₁ hybrid males examined for testes weight (T) and those examined for sperm counts (S).

§Mean number of sperm heads in paired epididymides.

plays several attributes of hybrid sterility¹⁵; the two homozygous parental forms, CBA/J and CBA-T6/T6, are normally fertile, the impairment of gametogenesis being limited only to T6 heterozygous male progeny of a CBA/J × CBA-T6/T6 cross. This combination is particularly interesting as the two parental strains, CBA/J and CBA-T6/T6 are genetically (quasi-)identical, except for a single reciprocal translocation between chromosomes 14 and 15. Thus, spermatogenic failure of the F₁ hybrids can be entirely ascribed to the heterozygosity for this chromosomal rearrangement.

Chromosome 17 of the mouse carries at least two gene loci involved in a similar type of male-limited sterility, that is, the sterility factor of *t* alleles^{16,17} and the *Hst-1* gene¹³. Using the *H-2* complex and *T* allele (*Brachyury*) as markers of chromosome 17, a distinct part of genetic variation in spermatogenic failure of T6 heterozygotes can be ascribed to a gene (or a gene cluster) linked to these markers on chromosome 17. The conclusion is drawn from an analysis of male progeny of CBA-T6/T6 × (C57BL/10-T × C3H)/+ cross. Those males that received, from their F₁ parent, the *H-2^b* haplotype and/or the *T* allele (the

two markers of chromosome 17 of C57BL/10-T strain) had significantly lower testes weights and sperm counts than their brothers with $H-2^k$ and $+^r$ markers of C3H origin (Fig. 1).

The relationship of the described gene to T/t and $H-2$ complexes and to the $Hst-1$ gene is not quite clear; it seems unlikely that the T allele itself would be involved since $T/+$ and $+/+$ male progeny of the CBA-T6/T6 $\times$ C57BL/10-T cross does not differ in testes weights. Furthermore, the strain distribution shown in Table 1 does not fit the assortment of alleles either of the $Hst-1$ gene¹³ or of the $H-2$ complex; in this case, however, the possibility cannot be excluded that the genetic background in some strains can alter the expression of the described gene.

Although the precise location of the described gene on chromosome 17 might have a bearing on the concept of the $T/t-H-2$ supergene¹⁸⁻²⁰, two other questions that emerged from this preliminary genetic analysis are those concerning the effect of the described gene on further male-sterile translocations, and the role of this gene in the presumed mechanism⁵ of translocation-associated male sterility.

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J. FOREJT

*Institute of Molecular Genetics,
Czechoslovak Academy of Sciences,
142 20 Prague 4, Czechoslovakia*

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¹ Lifschytz, E., in *Proc. int. Symp. Genet. Spermatozoon* (edit. by Beatty, R.A., and Gluecksohn-Waelsch, S.), 223-232 (University of Edinburgh, Edinburgh 1972).

² Lifschytz, E., and Lindsley, D. L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 182-186 (1972).

³ Epstein, C. J., *Science*, **163**, 1078 (1969).

⁴ Gartler, S. M., Liskay, R. M., and Gant, N., *Expl Cell Res.* **82**, 464-466 (1973).

⁵ Forejt, J., *Cytogenet. Cell Genet.*, **13**, 369-383 (1974).

⁶ Lyon, M. F., and Meredith, R., *Cytogenetics*, **5**, 335-354 (1966).

⁷ Cacheiro, N. L. A., Russell, L. B., and Swartout, M. S., *Genetics*, **76**, 73-91 (1974).

⁸ Searle, A. G., *Genetics*, **78**, 173-186 (1974).

⁹ Boer, P., de, and Groen, A., *Cytogenet. Cell Genet.*, **13**, 489-510 (1974).

¹⁰ Carter, T. C., Lyon, M. F., and Phillips, R. J. S., *J. Genet.*, **53**, 154-166 (1958).

¹¹ Bishop, D. W., in *The Testis* (edit. by Gomes, W. R., and Vandermark, N. L.), 41-61 (Academic, London and New York, 1970).

¹² Cattanaach, B. M., and Moseley, H., *Cytogenet. Cell Genet.*, **12**, 264-287 (1973).

¹³ Forejt, J., and Iványi, P., *Genet. Res.*, **24**, 189-206 (1975).

¹⁴ Searle, A. G., and Beechey, C. V., *Mutat. Res.*, **22**, 63-72 (1974).

¹⁵ Dobzhansky, T., *Genetics and the Origin of Species* (Columbia University, New York, 1951).

¹⁶ Dunn, L. C., *Science*, **144**, 260-263 (1964).

¹⁷ Lyon, M. F., and Meredith, R., *Hereditas*, **19**, 313-325 (1964).

¹⁸ Snell, G. D., *Folia Biol.*, **14**, 335-358 (1968).

¹⁹ Iványi, P., *Curr. Top. Microbiol. Immun.*, **53**, 1-90 (1970).

²⁰ Artzt, K., and Bennett, D., *Nature*, **256**, 545-547 (1975).

²¹ Stimpfling, J. H., *Transplant. Bull.*, **27**, 109-111 (1961).

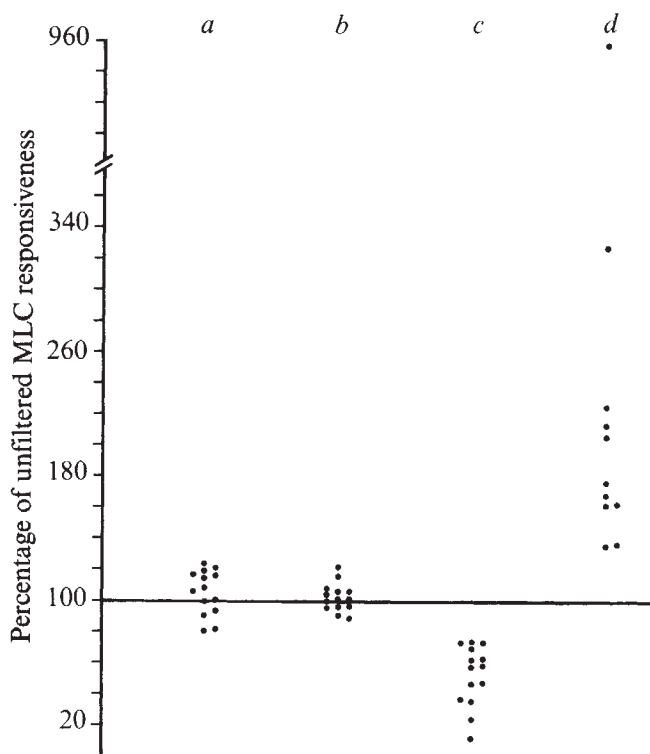
lymphocytes from this column was 93%, and the viability was greater than 99% by Trypan blue exclusion. Thus, the only discernible effect of this procedure was selectively to remove adherent cells which ingest latex particles.

Using well defined methods⁷, one-way MLCs involving 5×10^4 responding cells and 5×10^4 X-irradiated stimulating cells were planted in microtrays in 150 μ l of RPMI-1640 containing 20% pooled normal human serum, HEPES buffer (25 mM), penicillin (100 U ml⁻¹), streptomycin (100 μ g ml⁻¹), and fresh glutamine (2 mM). In all experiments, only fresh cells were used. MLCs were cultured for 132 h in a humidified atmosphere of 5% CO₂ and were labelled for the last 16 h of culture with 2.0 μ Ci of ³H-thymidine. Cultures were collected on to glass-fibre filters with a semi-automated sample harvester, and ³H-thymidine incorporation was determined by liquid scintillation spectroscopy. The incorporation of isotope was expressed as the mean c.p.m. of triplicate allogeneic cultures minus the mean c.p.m. of the isogenic control.

Figure 1 clearly demonstrates that removal of adherent latex-ingesting cells either isogenic to the stimulator or isogenic to the responder has no significant effect on MLC reactivity ($P > 0.20$ by two-tailed t test). Removal of adherent cells from both the stimulating and responding cell populations, however, results in significantly decreased MLC reactivity ($P > 0.05$ by one-tailed t test), in spite of the fact that the relative proportions of T and B lymphocytes in the column-filtered cells were virtually unchanged. It must be noted that these MLCs involved allogeneic combinations of HLA dissimilar individuals who were mutually strongly responsive in the routine MLC.

Abnormally low responsiveness in MLC can be quite

Fig. 1 *a-c*, The response of filtered cells of 14 normal individuals in MLC. Each point represents the mean of 3 separate MLCs and is expressed as the percentage of the mean of the 3 companion unfiltered MLCs. Response of a single individual's filtered cells to 3 separate allogeneic stimuli is 81-124% of the unfiltered response (*a*). The mean response of 3 separate individuals' cells to a single filtered allogeneic stimulus is 88-121% of the unfiltered response (*b*). The mean response of a single filtered cell population to 3 separate filtered allogeneic stimuli is attenuated (*c*). The increased responsiveness of patients' filtered lymphocytes to 4 separate allogeneic stimuli relative to the unfiltered responses (*d*).



Facilitation or attenuation of mixed leukocyte culture responsiveness by adherent cells

It is generally agreed that adherent cells, or macrophages, are required for maximum reactivity in mixed leukocyte culture (MLC). The system has been incompletely defined, for the mode of function of the adherent cell is unclear. There is evidence from human and animal systems that adherent cells have either a nonspecific helper role¹⁻⁴ or a more precise genetically defined stimulator role⁵. We report here that adherent cells which ingest latex particles seem to be capable of nonspecifically facilitating or attenuating human lymphocyte proliferation in MLC.

Heparinised venous blood was subjected to Ficoll-Hypaque flotation to obtain mononuclear cells. An aliquot of these cells was filtered through columns of Sephadex G-10 beads according to the method of Ly and Mishell⁶, the principal modification being the use of medium containing 20% foetal calf serum for elution. This procedure selectively depletes the mononuclear cells of adherent latex-ingesting cells while leaving the relative proportion of T and B lymphocytes unchanged (Table 1). In these experiments, we accounted for 88-103% of the mononuclear cells using conventional cell marker techniques. The mean yield of

Table 1 Cell marker analysis of filtered and unfiltered cells

Subject	Lymphocytes identifiable as T cells (%)		Lymphocytes identifiable as B cells (%)		Mononuclear cells identifiable as macrophages (%)	
	Unfiltered	Filtered	Unfiltered	Filtered	Unfiltered	Filtered
1	68	75			8.0	0
2	70	74			4.5	0
3	80	74			5.5	0
4	66	73			5.5	0.25
5			6	4	6.0	0
6			16	18	5.5	0
7			15	11	16.5	0
8			14	12	11.0	0
9			10	9	13.0	0
10	72	78	20	14	6.5	0.5
11	83	82	18	13	3.7	0
12	88	88	9	6.6	11.5	0
13	77	75	15	15.5	9.0	0
14	85	80	11	8	7.0	0
15	87	80	12	10	8.5	0.7

All percentages represent a minimum of 300 cells counted. T lymphocytes were determined by their ability to rosette spontaneously with sheep red blood cells. B lymphocytes were enumerated with a rhodamine-conjugated polyvalent anti-human immunoglobulin antiserum. Macrophages were identified by their ability to phagocytose latex particles 0.801 μ m in diameter.

reliably identified by calculating a relative response (RR)⁸ for an individual being tested. This value expresses the responsiveness of an individual's cells to a panel of four separate allogeneic stimuli, relative to the responses of several normal individuals to the same allogeneic panel. We have determined an RR for each of 33 normal individuals. The range was 79–136% with a geometric mean RR of 101%. An abnormally low RR was considered to be one greater than two standard deviations (2σ) below this mean (μ), namely, less than 76%. These calculations involved logarithmic transformations of the data since expression of these values as relative changes, by definition, generated a log normal distribution.

Eleven individuals demonstrating abnormally low responses in MLC were selected from a group of patients with non-lymphoid malignancies. Six of these individuals demonstrated RRs $>3\sigma$ ($<66\%$) below μ , and the other five demonstrated RRs $\geq 5\sigma$ ($<50\%$) below μ . Figure 1 shows that filtered lymphocytes from these individuals, which were virtually devoid of adherent latex-ingesting cells, demonstrated significantly increased proliferative capacities in response to each of four normal allogeneic stimuli compared with the companion MLCs using unfiltered patient responding cells. When column-filtered lymphocytes were used, the RRs of eight of these individuals returned to values within the normal range as defined above, while the remaining three returned to near normal (data not shown). This markedly increased responsiveness was significantly different from that observed with 56 normal allogeneic combinations involving filtered responding cells ($P < 0.005$ by one-tailed t test). In addition, four of these patients demonstrated a significantly decreased ability to stimulate normal allogeneic cells. When the patients' adherent cells were removed from the stimulating population by column filtration, the proliferative capacity of the four normal responders returned to normal values.

Our findings with mononuclear cells of HLA dissimilar normal individuals confirm the findings of others^{2,9,10} that adherent cells are necessary for optimal MLC responsiveness whether the adherent cell is isogenic or allogeneic to the responding cell population. Depletion of these cells from both the stimulating and responding cell populations results in significantly decreased MLC responsiveness. In addition, in the pathological situation, the adherent cell can be responsible for attenuated lymphocyte blastogenic activity, and when removed from culture, normal or near normal lymphocyte proliferation may be observed. Our experiments strongly suggest that such increased proliferation of patient lymphocytes is mediated by the allogeneic

adherent cells in the normal irradiated stimulating cell population, giving further testimony to the nonspecific effects which mononuclear phagocytes may display in MLC

In the light of the findings that macrophages can secrete discrete factors which are either stimulatory or inhibitory for cellular proliferation¹¹, it is appropriate to speculate that in human malignancy a perturbation of macrophage function occurs which involves a net increase of an inhibitory influence during the response to an allogeneic stimulus.

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N. T. BERLINGER

C. LOPEZ

R. A. GOOD

Memorial Sloan-Kettering Cancer Center,
1275 York Avenue,
New York, New York 10021

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- Gordon, J., *Proc. Soc. exp. Biol. Med.*, **127**, 30–33 (1968).
- Twomey, J. J., Sharkey, O., Jr., Brown, J. A., Laughter, A. H., and Jordan, P. H., Jr., *J. Immunol.*, **104**, 845–853 (1970).
- Levis, W. R., and Robbins, J. H., *Transplantation*, **9**, 515–518 (1970).
- Rode, H. N., and Gordon, J., *Cell. Immunol.*, **13**, 87–94 (1974).
- Greineder, D. K., Rosenthal, A. S., *J. Immunol.*, **114**, 1541–1547 (1975).
- Ly, I. A., and Mishell, R. I., *J. Immun. Meth.*, **5**, 239–247 (1974).
- Jorgenson, F., and Lamm, L. U., *Tissue Antigens*, **4**, 482–494 (1974).
- Thorsby, E., et al., *Tissue Antigens*, **4**, 507–525 (1974).
- Rode, H. N., and Gordon, J., *J. Immunol.*, **104**, 1453–1457 (1970).
- Alter, B. J., and Bach, F. H., *Cell. Immunol.*, **1**, 207–218 (1970).
- Calderon, J., and Unanue, E. R., *Nature*, **253**, 359–361 (1975).

Isolation of a subpopulation of adherent peritoneal cells with anti-tumour activity

We have reported that adherent peritoneal cells from normal mice stimulated the growth of many lymphomas *in vitro*, while adherent peritoneal cells from mice injected with Bacille Calmette-Guerin (BCG) were far less stimulatory¹. The latter were overtly cytostatic when tested on L929 cells¹, as well as on several other adherent fibroblastic malignant targets (unpublished). This leads us to speculate that adherent murine peritoneal cells consist of at least two subpopulations, one that tends to stimulate and one that tends to inhibit the proliferation of susceptible target cells. According to this view, infection of the mouse with BCG might lead to increased numbers or activity of the cytostatic subpopulation at the expense of the stimulatory subpopulation. We have now isolated an adherent, esterase-positive, poorly phagocytic mononuclear peritoneal

cell with potent tumour-inhibitory capacity, which may represent the postulated cytostatic subpopulation.

Cells were washed from the unstimulated peritoneal cavities of DBA/2 or CDF1 mice or from age-matched mice 1–21 weeks after intraperitoneal injection of 10^7 viable BCG of the Phipps or Pasteur strains (Trudeau Nos 1029 or 1011). The cells were washed and allowed to adhere to plastic 35-mm dishes (Falcon) for 2–3 h in RPMI-1640 with 20% foetal calf serum. The dishes were then rinsed vigorously in beakers of saline, leaving cells which were essentially all esterase positive¹. Starch granules prepared from the seeds of *Amaranthus caudatus*² were added to the monolayers in conditions leading to maximal phagocytic uptake, as defined for these cell preparations in preliminary experiments with ¹⁴C-acetyl-labelled granules by methods described previously^{3,4}. At the conclusion of the phagocytic period, the dishes were again washed vigorously in six beakers of saline.

In conditions of maximal uptake (15–20 mg of starch per dish for 3 h), microscopy revealed that $4.8 \pm 0.5\%$ of the adherent peritoneal cells in the monolayers from normal mice and $16.8 \pm 1.3\%$ of those from BCG mice were non-phagocytic for starch (mean $\pm$ s.e. for 14 and 17 experiments, respectively; "non-phagocytic" defined as 0–1 cell-associated granule). Cells were then dislodged from the monolayers by incubation in medium containing lidocaine⁵ (Astra Pharmaceuticals) followed by exposure to jets of saline from a syringe. This procedure recovered nearly all cells from the normal monolayers but left behind a variable number of non-phagocytic cells in the dishes prepared from BCG-treated mice. The cells were washed, spun on Ficoll-Hypaque⁶ at 240g for 12 min, and the non-sedimenting cells were washed again. Phagocytic cells and free starch pelleted, leaving at the interphase a preparation of non-phagocytic mononuclear cells with a viability of $92.4 \pm 1.9\%$ by Trypan blue exclusion, contaminated with $3.5 \pm 1.3\%$ of viable cells containing more than one starch granule, and with $0.009 \pm 0.0004\%$ of the initial free starch granules (mean $\pm$ s.e. from 14 to 17 preparations).

These non-phagocytic cells were plated in microtest wells (Falcon) in 0.2 ml with various tumour cells for 2 d, as indicated in Table 1. Uptake of ³H-thymidine during the last 4 h of culture was measured as described before¹. In each of five experiments, the isolated non-phagocytic adherent cells inhibited the uptake of ³H-thymidine by the tumour targets to a greater degree than similar numbers of the total adherent peritoneal cell populations from which they were derived. The non-phagocytic adherent cells from BCG-treated mice appeared to differ from those from normal mice only in that there were nearly four times as many of them, and they were apparently

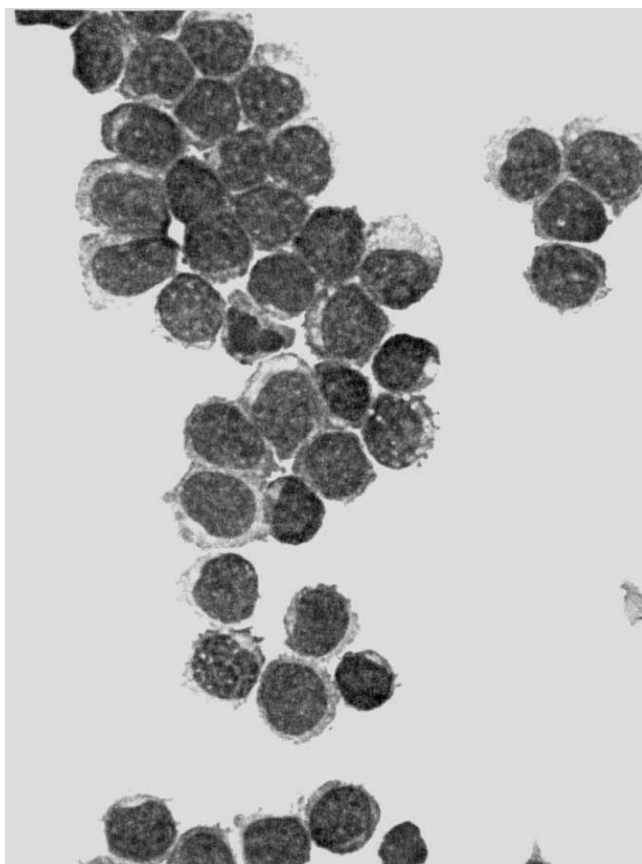


Fig. 1 Cytocentrifuge preparation of non-phagocytic adherent peritoneal cells prepared from untreated mice, as described in the text. (Wright's stain; magnification $\times 880$.)

more resistant to dislodgement by lidocaine. In dose-response experiments (not shown), there was no evidence that, per recovered cell, they were more potent. As few as 2.5×10^3 – 5×10^3 cells of the non-phagocytic subpopulation from either the BCG or the normal adherent populations markedly inhibited the uptake of ³H-thymidine by 1×10^3 MCA-3 cells. Controls showed that the addition of starch and lidocaine to peritoneal cells in suspension with rapid centrifugation over Ficoll-Hypaque, before phagocytosis had proceeded very far, gave preparations which behaved like the parent populations (Experiment 2, Table 1). Likewise, when peritoneal cells were

Table 1 ³H-thymidine uptake after 2-d culture of tumour cells with adherent peritoneal cells (c.p.m., mean $\pm$ s.e.†)

Experiment	Tumour*	No. of tumour cells plated	Tumour alone	With total adherent cells‡		With subpopulation‡¶	
				Normal	BCG	Normal	BCG
1	MCA-2	1×10^3	$2,310 \pm 87$	$10,229 \pm 1,017$	$1,332 \pm 103$	ND	0 ± 141
		5×10^3	$1,280 \pm 104$	$5,847 \pm 559$	651 ± 157	ND	0 ± 50
2	MCA-3	1×10^3	$4,996 \pm 158$	$5,766 \pm 492$	$2,976 \pm 376$	319 ± 13	267 ± 54
				$(7,476 \pm 527)\S$	$(3,266 \pm 151)\S$		
3	MCA-3	1×10^3	$1,999 \pm 84$	$4,319 \pm 463$	$1,480 \pm 37$	29 ± 8	99 ± 15
		1×10^3	$4,020 \pm 153$	$7,393 \pm 462$	$2,744 \pm 66$	74 ± 15	25 ± 6
4	MCA-3	1×10^3	$1,567 \pm 25$	$3,121 \pm 334$	$1,134 \pm 49$	181 ± 24	329 ± 31
		5×10^3	$10,070 \pm 896$	$15,352 \pm 897$	$4,495 \pm 516$	$1,243 \pm 71$	ND
5	MCA-3	1×10^3	$2,507 \pm 85$	$2,935 \pm 310$	$1,418 \pm 39$	277 ± 65	381 ± 46
		5×10^3	$7,525 \pm 48$	$13,200 \pm 533$	$3,679 \pm 254$	804 ± 103	778 ± 43

* MCA-2 and MCA-3 are tissue culture lines derived from methylcholanthrene-induced sarcomas of the C57BL/6 mouse. L929 cells were obtained from Microbiological Associates.

† c.p.m. are corrected for the c.p.m. taken up by each group in the absence of tumour cells. Mean background values for each column from left to right above were: 33 ± 5 (no cells; machine background); 291 ± 109 ; 120 ± 66 ; 45 ± 10 ; 42 ± 18 .

‡ For total adherent cells, 5×10^4 peritoneal cells were added to each well for 3 h and non-adherent cells were removed before addition of tumour cells, leaving an average of 2×10^4 adherent peritoneal cells¹. For the subpopulation, 2×10^4 cells were used in each well. Values are for triplicate cultures.

§ Peritoneal cells treated with lidocaine and starch in suspension before being processed over Ficoll-Hypaque.

¶ After phagocytosis, monolayers were treated with 36 mM lidocaine for 2 min (Experiments 1 and 2) or 12 mM lidocaine for 5 min (Experiments 3 and 5). The latter gave better yields.

allowed to adhere, collected with lidocaine and processed over Ficoll-Hypaque exactly like the experimental cells, except for the omission of starch, they interacted with tumour cells just like the parent peritoneal cell populations. These results suggest that the isolation procedure itself did not induce anti-tumour activity. In addition, anti-tumour activity could not be induced by addition of contaminating amounts of free starch to wells containing the tumour alone or tumour with either adherent peritoneal cells or the non-phagocytic subpopulation. There was no inhibition of uptake of ^3H -thymidine in MCA-3 cells added to cultures of non-phagocytic adherent cells which had been allowed to die by 4–5 d of preculture. Inhibition of uptake of ^3H -thymidine accurately reflected changes in viable tumour cell number as judged by haemocytometer counts.

Like the total adherent cell population from which they were derived¹, the isolated non-phagocytic adherent cells were esterase positive. Between 0.3 and 10% of non-phagocytic adherent cells from BCG-treated mice seemed to contain acid-fast organisms by Ziehl-Neelsen staining, compared with 3–10% of the total adherent peritoneal cells from such mice (cells from normal mice showed no positive staining). Because of the rare stainable intracellular mycobacteria and the fact that 10–50% of the "non-phagocytic" adherent cells (as operationally defined) seemed to contain one starch granule each, it may be preferable to regard these cells as "poorly phagocytic". When Wright's stain was used (Fig. 1), the active subpopulation comprised cells intermediate in size between small lymphocytes and macrophages, with scant to moderate, pale blue, sometimes vacuolated cytoplasm, a spiculated or ruffled cell margin, and an oval to reniform nucleus, occasionally with prominent nucleoli. Further work is in progress to characterise these cells; it is not clear whether they are macrophages.

Allison described non-phagocytic, adherent murine peritoneal exudate cells which he suggested might be effector cells in antibody-dependent cell-mediated cytotoxicity⁷. Cells with some similar characteristics were found in mouse spleen by Greenberg *et al.*⁸. Boyle and Ormerod isolated a subpopulation of phagocytic, adherent peritoneal cells from alloimmunised mice which seemed to bear all the cytotoxic potential for the specific tumour cell target⁹. Subpopulations of macrophages with different activities in endocytosis^{10,11}, immune RNA production¹¹, or antigen binding¹² have been obtained using density centrifugation in Ficoll^{10,12} or albumin¹¹. It is not yet known whether there is any relationship between the subpopulations described by these workers and the subpopulation described here.

Because of the metabolic perturbations induced by phagocytosis itself^{13,14}, the separation procedure used here precludes testing the residual, phagocytic cells in their basal state. Other techniques will be needed before the non-phagocytic adherent subpopulation can be compared directly with the remainder of adherent peritoneal cells in an anti-tumour assay.

Work is in progress to test the hypothesis that different classes of tumour cells differ in their susceptibility to the postulated inhibitory and stimulatory subpopulations of adherent peritoneal cells.

Thus, among adherent murine peritoneal cells, there are esterase-positive cells which can be isolated in nearly pure form on the basis of their deficient phagocytosis. These cells markedly inhibit the replication of various tumour cells *in vitro*, and seem to account quantitatively for the inhibition afforded by the total adherent peritoneal cell population from which they were derived. The tumour-inhibitory subpopulation can be isolated from untreated mice, although it can be identified in greater number among the adherent peritoneal cells from mice given BCG. The anti-tumour action of the subpopulation seems to be nonspecific. The existence of such a subpopulation may necessitate reassessment of the relationship of the numerous features of "macrophage activation", measured in a whole population, to its anti-tumour activity.

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CARL F. NATHAN
VIRGINIA M. HILL
WILLIAM D. TERRY

*Immunology Branch,
National Cancer Institute,
National Institutes of Health,
Bethesda, Maryland 20014*

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- ¹ Nathan, C. F., and Terry, W. D., *J. exp. Med.*, **142**, 887–902 (1975).
- ² MacMasters, M. M., Baird, P. D., Holzapfel, M. M., and Rist, C. E., *Econ. Bot.*, **9**, 300–302 (1975).
- ³ Michell, R. H., Pancake, S. J., Noseworthy, J., and Karnovsky, M. L., *J. Cell Biol.*, **40**, 216–224 (1969).
- ⁴ Nathan, C. F., Karnovsky, M. L., and David, J. R., *J. exp. Med.*, **133**, 1356–1376 (1971).
- ⁵ Rabinovitch, M., and DeStefano, M., *In Vitro* **11**, 379–381 (1975).
- ⁶ Böyum, A., *Scand. J. clin. lab. Invest.*, **21**, Suppl., 97, 77–89 (1968).
- ⁷ Allison, A. C., *Ann. Inst. Pasteur*, **123**, 585–608 (1972).
- ⁸ Greenberg, A. H., Shen, L., and Roitt, I. M., *Clin. exp. Immun.*, **15**, 251–259 (1973).
- ⁹ Boyle, M. D. P., and Ormerod, M. G., *Cell. Immun.*, **17**, 247–258 (1975).
- ¹⁰ Zembla, M., and Asherson, G. L., *Immunology*, **19**, 677–681 (1970).
- ¹¹ Rice, S. G., and Fishman, M., *Cell. Immun.*, **11**, 130–145 (1974).
- ¹² Walker, W. S., *Immunology*, **26**, 1025–1037 (1974).
- ¹³ Sbarra, A. J., and Karnovsky, M. L., *J. biol. Chem.*, **234**, 1355–1362 (1959).
- ¹⁴ Clark, R. A., Klebanoff, S. J., Einstein, A. B., and Fefer, A., *Blood*, **45**, 161–170 (1975).

Inhibition and reversal of wheat germ agglutinin-induced caps by concanavalin A

ASSEMBLY of cell-surface components at one pole of the cell membrane, as a result of cross linkage with specific ligands, can be visualised by using labelled ligands. Such a demonstration of topographic changes on the cell surface is called capping. By using different labels for the different ligands it is possible to study the mutual relationship between various membrane components. Using the capping technique, it was possible to demonstrate the relationship between concanavalin A (con A) receptors and immunoglobulin (Ig) receptors on the lymphocyte surface¹, as well as other surface antigens such as H-2, HLA and θ antigens present on murine and human lymphocytes^{2–4}. The present study was undertaken to demonstrate the relationship between the receptors of two plant lectins, con A with specificity for glucoside residues, and wheat germ agglutinin (WGA) with specificity for N-acetyl-glucosamine residues⁵, that are both present on the surface of murine thymocytes.

Thymocytes of 4–6-week-old C57BL/6 mice were used in all experiments. Con A, WGA, con A labelled with rhodamine (Rh-con A) and WGA labelled with fluorescein (Fl-WGA) were obtained from Miles-Yeda. Succinyl-con A was prepared in our laboratory according to Gunther *et al.*⁶.

Incubation of 5×10^6 thymocytes per ml with Fl-WGA, Rh-con A or with both lectins simultaneously, induced capping on the cells. Table 1 *a–d* shows the results of such experiments. It can be seen that Fl-WGA induced cap formation in 91% of the cells, while Rh-con A only in 7%. When cells were incubated with Fl-WGA and unlabelled con A or with Fl-WGA and Rh-con A, however, only 2% of the cells showed caps to WGA, whereas 89% of the cells showed a uniform distribution of the Fl-WGA around the cell surface. The presence of con A inhibited cap formation induced by Fl-WGA (Fig. 1c). Addition of succinyl-con A did not cause such an inhibitory effect (Table 1e). Pretreatment of the thymocytes with 10^{-4} M colchicine affected con A capping, as can be seen in Table 1f–h. This pretreatment elevated the percentage of cells with caps to Rh-con A from 7 to 22. Such pretreatment also elevated the percentage of caps to Fl-WGA from 2 to 19% in cells simultaneously incubated with con A. Moreover, the location on the cell surface of Fl-WGA caps was identical to the location of Rh-con A caps (Fig. 1d). The same phenomenon was also observed when Rh-con A treated cells were washed, incubated

Table 1 Inhibition and cocapping of WGA receptors by con A

Treatment of thymocytes	Distribution of WGA receptors			Distribution of con A receptors		
	% caps	% uniform	% unlabelled	% caps	% uniform	% unlabelled
a FI-WGA	91	0	9	NT	NT	NT
b Rh-con A	NT	NT	NT	7	93	0
c FI-WGA+con A	2	89	9	NT	NT	NT
d FI-WGA+Rh-con A	2	89	9	6	94	0
e FI-WGA+succinyl-con A	88	2	10	NT	NT	NT
f Colchicine+Rh-con A	NT	NT	NT	22	78	0
g Colchicine+con A+FI-WGA	19	71	10	NT	NT	NT
h Colchicine+Rh-con A+FI-WGA	19	71	10	22	78	0
i Colchicine+Rh-con A+NaN ₃ +FI-WGA	20	72	8	24	76	0

Washed C57BL/6 thymocytes (5×10^6 cells per ml) were incubated for 15 min at 37 °C, in the following conditions: a, 24 µg FI-WGA; b, 50 µg Rh-con A; c, 24 µg FI-WGA+50 µg con A; d, 24 µg FI-WGA+50 µg Rh-con A; e, 24 µg FI-WGA+50 µg succinyl-con A; f, as in b with pretreatment of cells with 10^{-4} M colchicine; g, as in c with pretreatment of cells with 10^{-4} M colchicine; h, as in d with pretreatment of cells with 10^{-4} M colchicine; i, as in h with incubation of cells in 10 mM NaN₃ before addition of FI-WGA.

for 5 min in a solution containing sodium azide (non-capping conditions), and then FI-WGA was added (Table 1i). These results resemble cocapping obtained with con A and anti-Ig on the surface of lymphocytes pretreated with colchicine⁷. In colchicine treated thymocytes the morphology of con A caps differs from that of WGA caps in that con A caps are elongated (Fig. 1b), whereas WGA caps are round (Fig. 1a). When cocapping occurs, however, FI-WGA caps assume the morphology of con A caps. Addition of con A to thymocytes preincubated with FI-WGA reversed WGA caps to a uniform distribution 30 min after the addition of con A (Table 2b) (Fig. 1e). This reversion of WGA caps was dependent on the con A concentration: addition of 50–12 µg con A ml⁻¹ induced a complete reversal, while smaller amounts (6 µg and less) decreased the extent of reversal. This decrease was proportional to the amount of con A used. Such a reversion of FI-WGA caps in thymocytes was also observed when con A was added at 4 °C, or when cells were treated with sodium azide (10 mM) before addition of con A (Table 2d and e). Thus, reversion of WGA

caps by addition of con A occurs even in conditions in which cap formation is usually inhibited⁸. These results resemble the reversion of con A caps by cytochalasin B which is similarly unaffected by sodium azide⁴. Reversion of WGA caps was not observed when succinyl-con A was added 30 min after WGA-induced caps were already formed (Table 2g).

Addition of α -methyl-D-glucoside (0.1 M) after reversion of WGA caps by con A resulted in the reappearance of WGA caps (Table 2c). Addition of antibodies to WGA after cap formation by this lectin, prevented the cap reversion caused by addition of con A (Table 2f). Moreover, when Rh-con A was added to thymocytes which had been incubated with WGA and subsequently with antibodies to WGA, it was possible to simultaneously demonstrate FI-WGA caps and uniform distribution of Rh-con A (Table 2f), (Fig. 1f). Thus, addition of a specific cross linkage agent of WGA (anti-WGA) overcomes the antagonistic effect of con A.

The data in the present study demonstrates a mutual relationship between WGA and con A receptors on the cell surface of murine thymocytes. Receptors cross linked with con A control the topographic distribution of WGA receptors. In the presence of con A not only is WGA capping inhibited, but existing WGA caps are reversed to a uniform distribution. Such reversion is not affected by treatments involving sodium azide or 4 °C, indicating a process that does not require energy. Such a reversion in WGA caps can be overcome by addition of antibodies to

Fig. 1 Inhibition and reversion of caps induced by WGA. The photographs were taken using a Zeiss Universal microscope (barrier filters were used for observations of fluorescein fluorescence and rhodamine fluorescence). a, Cell stained with FI-WGA at 37 °C for 15 min; b, cell stained with Rh-con A at 37 °C for 15 min in the presence of 10^{-4} M colchicine; c, cell stained with Rh-con A and FI-WGA at 37 °C for 15 min. Both lectins were added simultaneously. Upper, Rh-con A; lower, FI-WGA. d, Cocapping of FI-WGA with Rh-con A in the presence of 10^{-4} colchicine. Upper, Rh-con A; lower, FI-WGA. e, Reversion of WGA caps by con A. A cell stained with FI-WGA, after 15 min Rh-con A was added for additional 30 min. Upper, Rh-con A; lower, FI-WGA. f, Cell stained with FI-WGA, after 15 min anti-WGA was added for 5 min, after which Rh-con A was added for additional 30 min. Upper, Rh-con A; lower, FI-WGA.

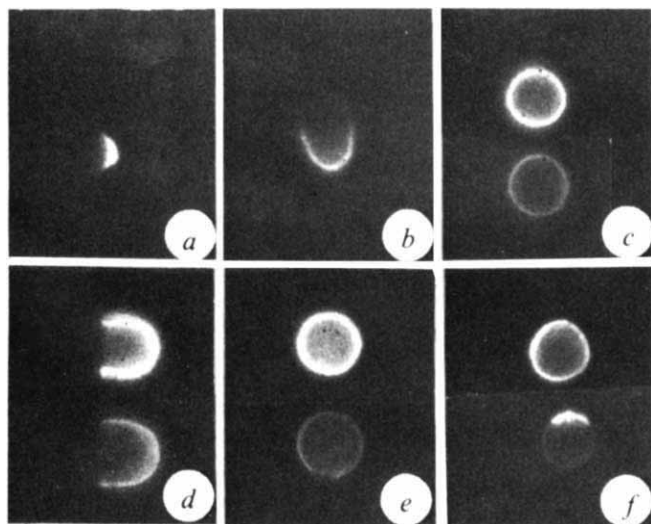


Table 2 Reversal of WGA caps by con A

Treatment of thymocytes	% WGA caps
a FI-WGA	91
b FI-WGA+con A	3
c FI-WGA+con A+ α -methyl-D-glucoside	91
d FI-WGA+NaN ₃ +con A	2
e FI-WGA+4 °C+con A	2
f FI-WGA+anti-WGA+con A	89
g FI-WGA+succinyl-con A	88

The cells were not washed after FI-WGA treatment, but identical results were obtained when cells were washed after this primary incubation. In experiment 2a 5×10^6 thymocytes per ml were incubated at 37 °C for 15 min in the presence of 24 µg FI-WGA. In experiment 2b 5×10^6 thymocytes per ml were preincubated at 37 °C for 15 min in the presence of 24 µg FI-WGA and then incubated with 50 µg con A at 37 °C for 30 min. In experiment 2c 5×10^6 thymocytes per ml were preincubated at 37 °C for 15 min in the presence of 24 µg FI-WGA, then incubated with 50 µg con A at 37 °C, after 30 min α -methyl-D-glucoside (0.1 M) was added at 37 °C for 15 min. In experiment 2d-f, 5×10^6 thymocytes per ml were preincubated at 37 °C for 15 min in the presence of 24 µg FI-WGA. Then the first sample was incubated with NaN₃ (10 mM) at 37 °C. The second at 4 °C. The third—with anti-WGA (5 µg Ab ml⁻¹)—at 37 °C. After 5 min 50 µg con A was added to the 3 samples for 30 min at the above-mentioned temperatures. In experiment 2g, 5×10^6 thymocytes per ml were preincubated at 37 °C for 15 min in the presence of 24 µg FI-WGA and then incubated with 50 µg of succinyl-con A for 30 min at 37 °C.

WGA, which probably increases the extent of cross linkage of WGA receptors.

Our results correlate with previously published studies dealing with the effect of con A on the capping of mouse lymphocyte surface immunoglobulin and on the capping of mouse thymocyte antigens^{1,4}. Our results could be interpreted by assuming that the surface molecule carrying con A receptors also carries WGA receptors as suggested for the case of con A and Ig receptors¹. This explanation is, however, not feasible for WGA and con A receptors since, in the presence of anti-WGA serum, WGA causes capping while con A does not. The WGA used in the present study is completely free of contaminating sugars, as assayed by the phenol-sulphuric acid test⁹, which precludes the possibility that con A binds to contaminated sugar residues present in the WGA preparation. Our data suggest the following alternative explanation: it is known that con A receptors of thymocytes and lymphocytes are a heterogeneous population in valence, affinity and chemical composition¹⁰ and are also known to be bound to microtubules which restrict their mobility⁴. In addition, the con A molecule is tetravalent¹¹ while WGA is divalent¹², thus enabling con A to form a firm lattice-network on the surface of the thymocytes. This con A receptor lattice may trap WGA receptors as well as other receptors such as Ig and θ antigens which also seem to be interspersed among con A receptors⁴. As a consequence the mobility of WGA receptors is governed by the lattice formed by con A, and therefore the WGA caps can be reversed by the denser lattice formed by con A when binding to its receptors.

Our explanation of the cap-reversion phenomenon is supported by the following two observations: (1) substitution of tetravalent con A with divalent succinyl-con A does not cause a reversion of previously formed WGA caps, and (2) addition of antibodies to WGA prevents con A reversion of WGA caps, apparently by increasing the extent of cross linkage of WGA receptors. Preliminary results in our laboratory have shown that the reversion phenomenon also occurs with Ig receptors by con A.

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SHLOMO LUSTIG
DOV H. PLUZNICK

Department of Life Sciences,
Bar-Ilan University,
Ramat-Gan, Israel

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- ¹ Yahara, I., and Edelman, G. M., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 608-612 (1972).
- ² Neaupoort-Sautes, C., Lilly, F., Silvestre, D., and Kourilsky, F. M., *J. exp. Med.*, **137**, 511-526 (1973).
- ³ Preud'homme, J. L., Neaupoort-Sautes, C., Piat, S., Silvestre, D., and Kourilsky, F. M., *Eur. J. Immun.*, **2**, 297-300 (1972).
- ⁴ De-Petris, S., *J. Cell Biol.*, **65**, 123-146 (1975).
- ⁵ Sharon, N., and Lis, H., *Science*, **177**, 949-959 (1972).
- ⁶ Gunther, G. R., Wang, J. L., Yahara, I., Cunningham, B. A., and Edelman, G. M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1012-1016 (1973).
- ⁷ Yahara, I., and Edelman, G. M., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1579-1589 (1975).
- ⁸ Loo, F., *Exp. Cell Res.*, **82**, 415-425 (1973).
- ⁹ Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F., *Analyt. Chem.*, **28**, 350-355 (1956).
- ¹⁰ Krug, V., Hollendberg, M. D., and Cuatrecasas, P., *Biochem. biophys. Res. Commun.*, **52**, 305-312 (1973).
- ¹¹ Edelman, G. M., Yahara, I., and Wang, J. L., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1442-1446 (1973).
- ¹² Levine, D., Kaplan, M. J., and Greenaway, P. J., *Biochem. J.*, **129**, 847-856 (1972).

Evidence for a soluble lymphocyte factor in the transplacental transmission of T-lymphocyte responses to *Mycobacterium leprae*

ANTIGENS introduced to the human foetus have been considered as "self" and thus not to provoke an immune response¹. But, no sudden changes in the immune mechan-

isms seem to coincide with birth, and the development of adult immune responses is gradual. Moreover, in sheep, antigens can give rise to more or less mature immune responses in the foetus^{2,3}, dependent on the stage of gestation. The nature of transmission of these immune responses from mother to foetus remains unclear. Brody *et al.*⁴ demonstrated in humans that T-lymphocyte sensitisation could be transmitted from mother to foetus and considered this was due to transplacental passage of antigen. Field and Caspary⁵ showed that cell-mediated responses can be induced in the foetus, but thought that either maternal lymphocytes or some subcellular lymphocyte factor crossed the placenta to sensitise the foetal lymphocytes. We have tested ten randomly selected normal mothers and their babies at parturition for evidence of sensitisation to antigens of *Mycobacterium leprae*. We found that when the mothers showed sensitisation their babies did too: the children of unsensitised mothers were not sensitised. We consider the likeliest explanation for our findings is the transplacental passage of a soluble lymphocyte factor.

Lymphocyte transformation tests (LTT) were carried out by a micromethod⁶ on both maternal and neonatal cord blood; whole washed *M. leprae* were used as antigen, and in each case the responses to BCG and PPD were also determined. Of the ten mothers tested, five showed a positive LTT response with *M. leprae* as antigen and five did not (Fig. 1). It was considered that a positive response existed when the LTT response with the antigen was more than

Fig. 1 LTT responses to whole *M. leprae* in mothers and neonates, comparing the five mothers with positive responses and the five mothers with negative responses (unstimulated control values are subtracted). ³H-TdR, Tritiated thymidine uptake.

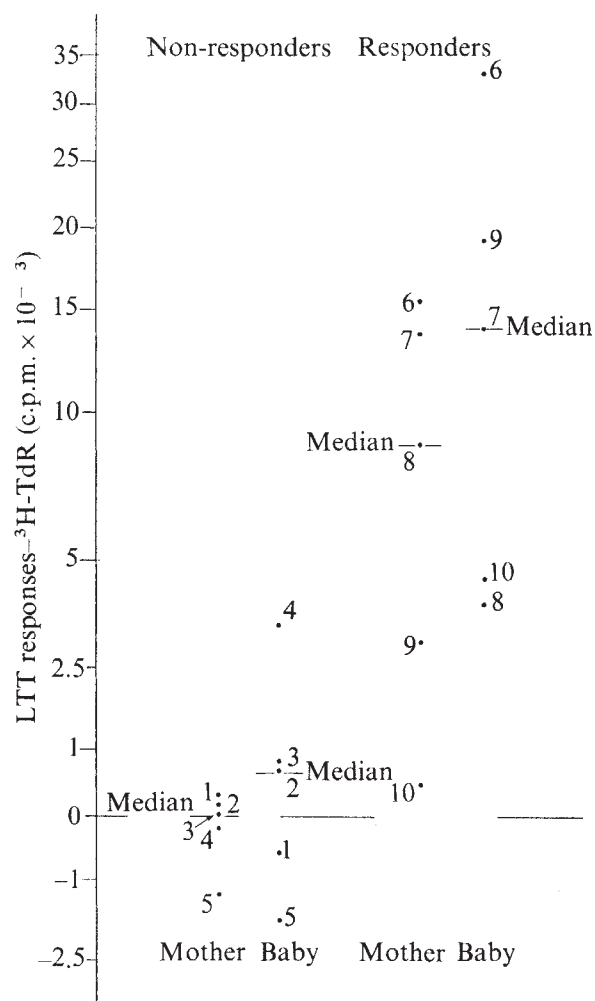


Table 1 Median responses to antigens

	<i>M. leprae</i>	BCG	PPD
Mothers with positive responses to <i>M. leprae</i>	8,600	267	4,316
Mothers with negative responses to <i>M. leprae</i>	-11	21	-36
Babies of mothers with positive responses to <i>M. leprae</i>	13,658	995	3,654
Babies of mothers with negative responses to <i>M. leprae</i>	602	-17	1,140

Figures are c.p.m.

Median responses to antigens in the two maternal and the two neonatal groups. *P*, Probability of distribution between the two groups occurring by chance, calculated by Mann-Whitney *U* test.

twice the value of unstimulated control cultures. The values for these control cultures were much higher in the neonates (median: 13,171) than in the mothers (median: 532). In the five mothers with positive lymphocyte blastogenic responses, the response of the neonate reflected the maternal response, though the former were much greater. In the five unresponsive mothers, the neonatal response was much lower than in the neonates born of responders. The difference between the two neonatal groups is highly significant ($P < 0.005$), using the Mann-Whitney *U* test for statistical analysis. There was also a statistically significant difference in responses to PPD (but not BCG) between the maternal responders and non-responders to *M. leprae* (Table 1), possibly due to some cross reactivity. There was, however, no significant difference of responses to PPD and BCG between the two neonatal groups.

Five out of ten may seem a high proportion of mothers to show evidence of sensitisation to *M. leprae*; but it is in accord with our experience that up to 90% of contacts with known cases of leprosy show evidence of previous sub-clinical infection⁷. Moreover, in Sao Paulo, Brazil, where the leprosy endemicity is similar to that of Addis Ababa, more than 50% of the normal population show positive lepromin (Fernandez) skin tests⁸.

This study provides evidence that when mothers are sensitised to *M. leprae* this sensitisation is transmitted to the foetus. The sensitisation seems to be specific, as there was no significant difference between the responses of the two neonatal groups when BCG or PPD were used as antigens.

For transmission of this sensitisation, there are three main possibilities. The first is transplacental passage of antigen. In our study, this seems unlikely as the mothers showed no signs of leprosy, or other mycobacterial disease. The second possibility is transplacental passage of maternal lymphocytes. It is still debatable whether maternal lymphocytes enter the foetal circulation⁹⁻¹², so if they do cross the placenta it must be in very small numbers. In view of the high neonatal LTT responses, recruitment of neonatal lymphocytes by maternal lymphocytes would again seem improbable. Third, there could be transplacental passage of a soluble lymphocyte factor. It seems most likely that such a factor was responsible for the foetal lymphocyte sensitisation we have demonstrated.

The nature of this lymphocyte factor remains unclear. Some immunoglobulins are known to cross the placenta, but none has been shown to raise antigen-specific T-lymphocyte responses in unsensitised individuals. Several soluble factors for specific T-cell stimulation however, have now been described. One possibility in this context is transfer factor¹³ which can transfer delayed type hypersensitivity and, having a molecular weight of less than

10,000, could cross the placenta passively. The nature of the factor suggested by our study clearly needs further investigation.

R. STC. BARNETSON

MRC Leprosy Project,

G. BJUNE

Armauer Hansen Research Institute,
Box 1005, Addis Ababa,

M. E. DUNCAN

Department of Obstetrics and Gynaecology,
National University, Addis Ababa, Ethiopia

Received November 21, 1975; accepted January 28, 1976.

- 1 Burnet, F. M., *Self and Non-Self* (Cambridge University Press, Cambridge, 1969).
- 2 Silverstein, A. M., Uhr, J. W., Kraner, K. L., and Lukes, R. L., *J. exp. Med.*, **117**, 799-812 (1963).
- 3 Silverstein, A. M., Prendergast, R. A., and Kraner, K. L., *J. exp. Med.*, **119**, 955-964 (1964).
- 4 Brody, J. I., Oski, F. A., and Wallach, E. E., *Lancet*, **i**, 1396-1398 (1968).
- 5 Field, E. J., and Caspary, E. A., *Lancet*, **ii**, 337-342 (1971).
- 6 Closs, O., *Infect. Immun.*, **11**, 1163-1169 (1975).
- 7 Godal, T., and Negasse, K., *Br. med. J.*, **3**, 557-559 (1973).
- 8 Rotberg, A., Bechelli, L. M., and Keil, H., *Int. J. Leprosy*, **18**, 209-220 (1950).
- 9 Desai, R. G., and Creger, W. P., *Blood*, **21**, 665-673 (1963).
- 10 Turner, J. H., Wald, N., and Quinlivan, W. L. G., *Am. J. Obstet. Gynec.*, **95**, 831-833 (1966).
- 11 Anderson, J. M., and Ferguson Smith, M. A., *Br. med. J.*, **2**, 166-167 (1971).
- 12 Olding, L., *Acta paediat. scand.*, **61**, 73-75 (1972).
- 13 Lawrence, H. S., *Proc. Soc. exp. Biol. Med.*, **71**, 516-522 (1949).

Cytoplasmic exclusion as a basis for asymmetric nucleocytoplasmic solute distributions

THE nuclear and cytoplasmic concentrations of solutes in cells are often unequal. This has been observed for ions^{1,2}, small molecules³⁻⁵, and for macromolecules⁶⁻¹⁰. The role of maintaining these concentration differentials is commonly assigned to the nuclear envelope, but this practice is predicated on a questionable analogy with the plasma membrane. Most of the information derived from tracer kinetics^{4,8,9} and electrical resistance¹⁰⁻¹³ indicates that the nuclear envelope is too permeable to distinguish or differentially transport small solutes. Thus, we must look to other, presumably equilibrium, mechanisms to account for many nucleocytoplasmic solute asymmetries. One useful step in the analysis of solute distributions between two phases is to establish a reference phase to which the concentrations of substances in the other two phases can be compared. A suitable reference phase for cytoplasm and nucleus cannot, of course, be separated from them by the cell membrane, which itself has complex, asymmetric transport properties. Thus, the reference phase must be intracellular. We have carried out experiments in which a defined aqueous reference phase (a gelatin gel) was introduced into the cytoplasm, and the partition of solutes between this phase, cytoplasm, and nucleus was determined. We deal here with the intracellular distribution of ³H-sucrose—a small, non-metabolised substance which reaches a greater concentration in the nucleus than in the cytoplasm⁴.

The experimental design was as follows: a solution of 11% fluorescein isothiocyanate-labelled gelatin was microinjected into the vegetal hemispheres of grown *Rana pipiens* oocytes at 34 °C. The oocytes were immediately cooled in ice-cold Ringer's to gel the gelatin, and then brought to 15 °C. The gelatin remained gelled at this temperature and provided the intracellular reference phase. Shortly before or after gelatin injections (the sequence did not influence the results), ³H-sucrose was microinjected into the oocyte cytoplasm. The oocytes were incubated for at least 3 h (adequate time for ³H-sucrose to achieve diffusional equilibrium⁴), quenched to liquid nitrogen temperature, sectioned at -50 °C, and the intracellular distribution of tracer determined quantitatively by ultra-low temperature autoradio-

graphy¹⁴. Figure 1 is a diagram of a typical autoradiographic section showing the nucleus and the gelatin within the cytoplasm, with the local grain densities superimposed.

Silver grains were uniformly distributed under the cytoplasm, confirming that the injected ³H-sucrose had reached diffusional equilibrium. Grain densities under the nucleus and gelatin were also uniform but were higher than under the cytoplasm—2.66 and 2.67 times greater, respectively. When the nucleus–cytoplasm (N–C) grain density ratio is corrected for the different water content of the two compartments (N–C=1.88, ref. 1), a ³H-sucrose partition coefficient of 1.41 is obtained for nucleus–cytoplasm. The water content of the intracellular gelatin gel phase is assumed to be that of the gelatin sol injected (89%), and the corrected ³H-sucrose partition coefficient for gelatin–cytoplasm is 1.35. (It is possible that the water content of the gelatin changed subsequent to injection. Even total replacement of the gelatin by water, however, would yield a coefficient no less than 1.20). Table 1 summarises our results.

Control data include nucleocytoplasmic partition coefficients in oocytes injected only with ³H-sucrose, and the

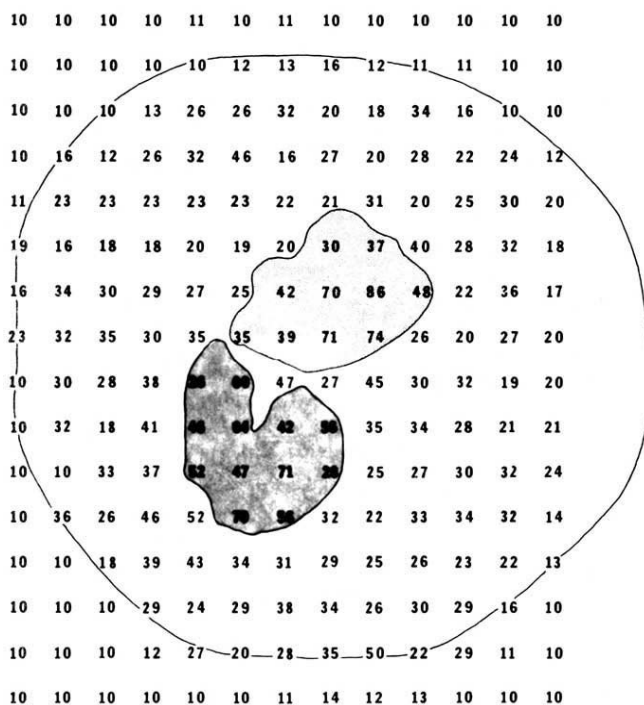


Fig. 1 Diagram of an ultra-low temperature autoradiographic section through the oocyte nucleus (light shading) and gelatin (dark shading). A volume of 11% fluorescein isothiocyanate-labelled gelatin about equal to that of the nucleus was microinjected as a sol (at 34 °C) in buffer (15 mM NaCl and 130 mM K phosphate, pH 7.1). After gelation (at 0 °C) of the gelatin, 0.2 μ Ci ³H-sucrose (New England Nuclear) in 5×10^{-5} ml water was microinjected and the oocyte incubated at 15 °C for a time sufficient for the ³H-sucrose to reach diffusional equilibrium (3–4 h). The oocyte was then rapidly embedded in OCT and quenched to –190 °C in liquid nitrogen, with sectioning and autoradiographic exposure carried out at ultra-low temperatures to prevent ³H-sucrose redistribution¹⁴. The nucleus was identified by its size, location in the animal hemisphere, absence of yolk platelets and pigment granules, and the presence of chromatin and nucleolar clumps. The gelatin appeared as an inclusion-free area which was positively identified by its fluorescence in blue light illumination. Superimposed on the section are relative local grain densities, determined photometrically with a Leitz MPV-1 microscope photometer using Ploem incident light illumination²². The grain density values shown are absolute photometer readings, and have not been corrected for the different water contents of the cytoplasmic, nuclear and gelatin compartments. The data summarised in Table 1 were derived from 18 similar two-dimensional grain-density plots from sections taken from five cells.

Table 1 Equilibrium partition of ³H-sucrose between aqueous phases at 15 °C

Phases	K*
Gelatin-injected cells	
Nucleus–cytoplasm	1.41 ± 0.03
11% Gelatin–cytoplasm	1.35 ± 0.03
Nucleus–11% gelatin	1.04
Controls	
Nucleus–cytoplasm (no gelatin injected)	1.34 ± 0.08
11% Gelatin–buffer (dialysis chamber)	0.98

* Ratio of ³H-sucrose concentrations in the water of the respective phases at diffusional equilibrium ($\pm$ s.e.m.).

dialysis partition coefficient between 11% gelatin and buffer. These controls show that intracellular gelatin does not affect the nucleocytoplasmic partition of ³H-sucrose. Furthermore, the ³H-sucrose asymmetries cannot be attributed to affinities of either gelatin or nucleus for ³H-sucrose, since the gelatin–buffer concentration ratio is essentially unity, as is that of nucleus–gelatin. We conclude that there must be an exclusion of ³H-sucrose from some of the cytoplasmic water—that is, cytoplasmic water is less available as a solvent than the water of gelatin, the nucleus, or buffer. The data may be explained if 4% of the nuclear water and 32% of the cytoplasmic water are unavailable as solvent for ³H-sucrose.

Nucleocytoplasmic concentration asymmetries in oocytes have been previously reported for glycerol³, unbound Na⁺ (ref. 2), α -aminoisobutyric acid⁵, inulin⁸, three molecular sizes of dextran⁹, and exogenous proteins^{7,15}. Also, endogenous oocyte proteins exhibit nucleocytoplasmic distributions greater than unity^{15,16}, although their distributions may be caused in part by mechanisms other than cytoplasmic exclusion (for example, specific binding to nuclear components or asymmetric nuclear envelope transport).

Many other types of cells show asymmetric nucleocytoplasmic distributions of solutes, but without experimental analysis of the type described here, cases of cytoplasmic exclusion cannot be distinguished from asymmetries caused by other mechanisms. Nevertheless, because of the nature of the solutes and the magnitudes of the asymmetries observed, we suggest that the intracellular distributions of colloidal gold in amoeba^{18,19} and of fluorescein-labelled exogenous proteins in salivary gland cells¹⁰ of *Chironomus* may reflect the operation of cytoplasmic exclusion. It is likely that the asymmetric nucleocytoplasmic equilibrium distribution of the shuttling proteins of amoeba¹⁷ is also explicable by cytoplasmic exclusion processes. Data from these diverse cell types suggest that the concentration of soluble cellular components in the nucleus occurs by cytoplasmic exclusion in many cells.

Intracellular concentration differences are not restricted to the nucleus, and other organelle–cytoplasm solute distributions may reflect the operation of cytoplasmic exclusion. Specifically, cytoplasmic exclusion may have a previously unexpected role in determining solute distributions between the cytoplasm, vacuoles, and the cisternae of the endoplasmic reticulum and the Golgi apparatus.

Our observations demonstrate the existence of cytoplasmic exclusion: they do not specify the molecular mechanism(s) responsible. It is clear, however, that much less cytoplasmic water is available as solvent than is measurable by subtraction of dry from wet weights. The operative mechanisms may include changes in bulk solvent properties of water through electrostriction, hydrophobic interactions, and solvent–polymer H bonding, and/or restriction of the accessibility of the water to solute through solvent microcompartmentalisation or polymer–solute repulsive interactions. Analogues for the behaviour of the cytoplasm are found in other macromolecular systems, such as solutions of

441.6–400 nm, reaching a value of less than 0.02 at the vaccinia virus²⁰, hyaluronic acid²¹, and the cross-linked dextrans used in chromatography.

Whether these or other mechanisms are responsible for the exclusion behaviour of cytoplasm, the process itself should not be overlooked in seeking the explanation for asymmetric distributions of solutes between the nucleus and cytoplasm.

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SAMUEL B. HOROWITZ
PHILIP L. PAINE

Laboratory of Cellular Physiology,
Department of Biology,
Michigan Cancer Foundation,
110 East Warren Avenue,
Detroit, Michigan 48201

Received December 8; accepted December 31, 1975.

- ¹ Century, T. J., Fenichel, I. R., and Horowitz, S. B., *J. Cell Sci.*, **7**, 5–13 (1970).
- ² Century, T. J., and Horowitz, S. B., *J. Cell Sci.*, **16**, 465–471 (1974).
- ³ Horowitz, S. B., and Fenichel, I. R., *J. gen. Physiol.*, **51**, 703–730 (1968).
- ⁴ Horowitz, S. B., *J. Cell Biol.*, **54**, 609–625 (1972).
- ⁵ Frank, M., and Horowitz, S. B., *J. Cell Sci.*, **19**, 127–139 (1975).
- ⁶ Paine, P. L., and Feldherr, C. M., *Expl Cell Res.*, **74**, 81–98 (1972).
- ⁷ Gurdon, J. B., *Proc. R. Soc.*, **B176**, 303–314 (1970).
- ⁸ Horowitz, S. B., and Moore, L. C., *J. Cell Biol.*, **60**, 405–415 (1974).
- ⁹ Paine, P. L., Moore, L. C., and Horowitz, S. B., *Nature*, **254**, 109–114 (1975).
- ¹⁰ Paine, P. L., *J. Cell Biol.*, **66**, 652–657 (1975).
- ¹¹ Ito, S., and Loewenstein, W. R., *Science*, **150**, 909–910 (1965).
- ¹² Loewenstein, W. R., and Kanno, Y., *J. gen. Physiol.*, **46**, 1123–1140 (1963).
- ¹³ Loewenstein, W. R., *Protoplasmatologia*, **2**, 26–34 (1964).
- ¹⁴ Horowitz, S. B., in *Methods in Cell Biology*, **8** (edit. by Prescott, D. M.) (Academic, New York, 1974).
- ¹⁵ Bonner, W. M., *J. Cell Biol.*, **64**, 421–430 (1975).
- ¹⁶ Feldherr, C. M., *Expl Cell Res.*, **93**, 411–419 (1975).
- ¹⁷ Legname, C., and Goldstein, L., *Expl Cell Res.*, **75**, 111–121 (1972).
- ¹⁸ Feldherr, C. M., *J. Cell Biol.*, **14**, 65 (1962).
- ¹⁹ Feldherr, C. M., *J. Cell Biol.*, **39**, 49–54 (1968).
- ²⁰ McFarlane, A. S., MacFarlane, M. B., Amies, C. P., and Eagles, G. H., *Br. J. exp. Path.*, **20**, 485–501 (1939).
- ²¹ Ogston, A. G., and Phelps, C. F., *Biochem. J.*, **78**, 827–833 (1960).
- ²² Rogers, A. W., *J. Microsc.*, **96**, 141–153 (1972).

Retinal sensitivity to damage from short wavelength light

A GROWING body of literature attests to the deleterious effects of long term exposure to light^{1–8}. To define more critically the differences between thermal and photochemical effects, we have exposed the retinae of rhesus monkeys to eight monochromatic laser lines from 1,064–441.6 nm. Thermal damage to the retina is to be expected for the 1,064-nm line since the photopigments are not involved and energy absorption takes place predominantly in the melanin granules of the pigment epithelium and the choroid. Although data on pathogenesis are not yet available, we found some interesting differences in retinal sensitivity in going from the near infrared to the blue wavelengths in the visible spectrum.

In a recent paper⁹ on solar retinitis in the rhesus monkey, we pointed out that a simulated solar exposure of 3 min duration produced a retinal lesion which could not be explained solely in terms of thermal injury, but which must cause some damage by a photochemical mechanism. We postulated some type of thermally enhanced photochemical effect similar to Noell's findings¹. It now seems possible to account for solar retinitis almost entirely in terms of the photochemical effects of the short wavelength components in the Sun's spectrum. Tso¹⁰ has reported on some patients who gazed voluntarily at the Sun for an hour before submitting to enucleation for malignant melanoma. An estimate of the maximum temperature on the retina using White's¹¹ mathematical model for solar observation yields approximately 1.5 °C above ambient for a 2-mm pupil. Whereas these patients suffered minimal visual acuity effects and obviously did not sustain retinal burns, the pathology after enucleation indicated damage to the retina which must have resulted from photochemical

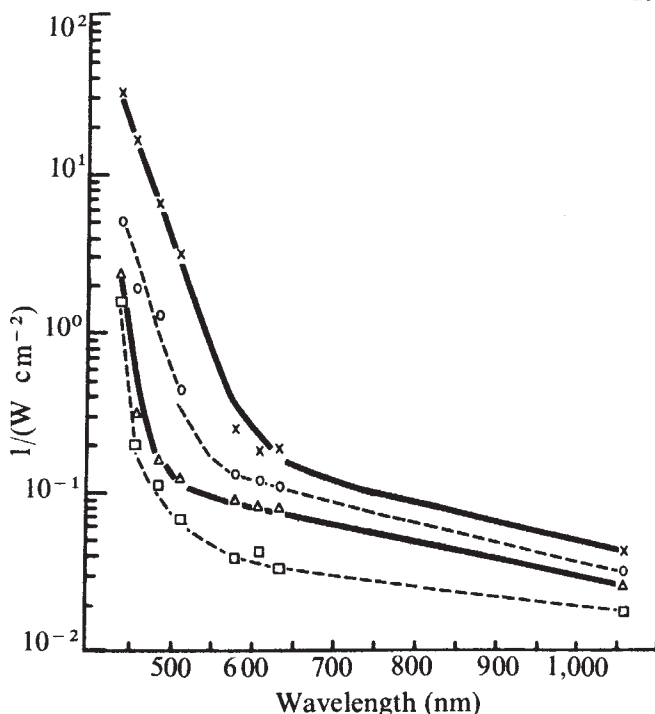


Fig. 1 Action spectrum of retinal sensitivity to threshold damage in the rhesus monkey. Reciprocal of retinal irradiance ($1/W \text{ cm}^{-2}$) is plotted semi-logarithmically against wavelength (nm) for exposure durations of 1, 16, 100 and 1,000 s. Beam diameter on the retina was 500 μm to the $1/e^2$ points of the Gaussian distribution. $\square$, 1s; $\triangle$, 16s; $\circ$, 100s; $\times$, 1,000s.

processes aided perhaps by thermal enhancement.

Results of the present experiments are summarised in Table 1, in which maximum retinal irradiance ($W \text{ cm}^{-2}$) and maximum temperature in the retina above ambient ($^{\circ}\text{C}$) are given for each exposure duration and wavelength. Maximum irradiance H_0 is based on the Gaussian distribution of the laser beam

$$H = H_0 \exp(-r^2/2\sigma^2)$$

where H is the irradiance at radius r from the Gaussian peak and σ is calculated from the beam profile by measuring r at the $1/e^2$ points. H_0 on the retina is calculated from the formula

$$H_0 = P_c T / 2\pi\sigma^2$$

where P_c is the power (W) entering the cornea and T the transmittance through the ocular media of the rhesus monkey as measured in our laboratory on nine eyes from five animals. An irradiance of $24 W \text{ cm}^{-2}$ at a temperature of approximately 23°C above ambient produced a threshold lesion in 1,000 s with the Nd-YAG laser (1,064 nm). In contrast, the 441.6-nm line from the He-Cd laser required only 30 mW cm^{-2} with negligible temperature rise to produce a threshold lesion in a 1,000-s exposure. The ratio of the two irradiances on the retina is 800. The Nd-YAG lesion has the typical appearance of a burn lesion, whereas the He-Cd lesion has no central core but appears as a slight patch of yellowish-white on the fundus, often with irregular boundaries. We attribute the lesion produced by 1,064-nm radiation to thermal effects, whereas that produced by the 441.6-nm line must be caused by photochemical processes.

Figure 1 is an action spectrum obtained by plotting semi-logarithmically the reciprocal of retinal irradiance ($1/W \text{ cm}^{-2}$) against wavelength (nm) for each exposure duration. The increase in retinal sensitivity with decrease in wavelength is steep even on a semi-logarithmic plot and shows no tendency to peak at 441.6 nm. Transmittance through the ocular media falls rapidly for wavelengths 441.6–400 nm, reaching a value of less than 0.02 at the

Table 1 Retinal irradiance H_0 (W cm^{-2}) $\pm$ s.d. for threshold lesion as function of wavelength (nm) and exposure duration (s)

Laser	Wavelength (nm)	Exposure duration (s)				Transmittance (T)
		1	16	100	1,000	
Nd-YAG	1,064	56.1 ± 5.3 55°	37.5 ± 4.2 37°	32.5 ± 3.1 32°	24.0 ± 3.0 23°	0.76
He-Ne	632.8	29.9 ± 1.4 49°	15.2 ± 3.0 25°	8.4 ± 2.8 14°	5.4 ± 2.3 9°	0.93
Ar-pumped dye laser	610	22.0 ± 1.9 36°	12.3 ± 1.1 20°	8.1 ± 1.1 13°	5.8 ± 0.3 9.5°	0.92
	580	26.1 ± 4.5 43°	11.5 ± 2.4 19°	7.6 ± 0.8 12.5°	4.0 ± 1.7 6.6°	0.91
Ar	514.5	14.5 ± 3.3 25°	10.3 ± 2.3 18°	2.2 ± 0.5 4°	0.32 ± 0.1 1°	0.87
Ar	488	9.4 ± 1.2 17°	6.1 ± 0.8 11°	0.77 ± 0.2 1°	$0.15 \pm .08$ 0.25°	0.83
Ar	457.8	5.1 ± 0.8 10°	3.2 ± 0.6 6°	0.52 ± 0.1 1°	$0.06 \pm .02$ 0.1°	0.69
He-Cd	441.6	$0.91 \pm .03$ 2°	$0.41 \pm .02$ 1°	$0.20 \pm .02$ 0.4°	$0.03 \pm .01$ 0.05°	0.45

Maximum retinal temperature ($^{\circ}\text{C}$) above ambient estimated from mathematical model of Clarke¹². T, Transmittance through ocular media. Beam diameter on retina 500 μm to $1/e^2$ points. Entire laser beam, TEM₀₀ mode, enters eye of anaesthetised animal with pupil dilated to 8 mm. A beam splitter and fundus camera are used to view and direct beam on the retina. Criterion for a threshold lesion is the appearance 24 h after exposure of a funduscopically visible lesion. For details of method of determining thresholds, see ref. 9.

lower wavelength. This is caused primarily by absorption in the lens. Whereas the sensitivity of the retina may continue to increase in the wavelength band 441–400 nm, the number of photons reaching the retina is reduced drastically by absorption and scattering in the lens and the rest of the ocular media. In fact, the threshold for damage may shift from the retina to cataractogenesis in the lens. We are attempting to explore this region of the spectrum using narrow bandpass filters with a 2,500-W xenon arc lamp. Also, it is planned to expose the retina of the monkey after surgical removal of the lens.

These results help to explain the phenomenon of solar retinitis which cannot be interpreted solely in terms of thermal injury. Unattenuated sunlight peaks at 470 nm and a relatively large proportion of the solar energy is concentrated in the blue end of the spectrum. For example, the solar retinal irradiance at 440 nm for a 20-nm spectral band is approximately 0.20 W cm^{-2} at midday for an eye gazing directly at the Sun at sea level with a 2-mm pupil. In comparison, the threshold irradiance for a 100-s exposure to the 441-nm laser line of He–Cd is 0.20 W cm^{-2} . Thus, sunbathing at bright midday for 100 s can produce a threshold lesion. It is our belief that solar retinitis is caused primarily by the short wavelengths in the visible spectrum of the Sun with some thermal enhancement from the longer wavelengths in the visible and near infrared. The retinal pathology among military personnel on a Pacific island as reported by Smith¹³ and the aetiology of foveomacular retinitis among military personnel as reported by Marlor¹⁴ can be better understood in terms of these results. Those subject to exposure to bright sunlight over long periods should take precautions to shield their eyes from the short wavelengths of solar radiation. This has implications in the design of sunglasses and welders' goggles. The sensitivity of the retina to short wavelengths of light may also have clinical significance for certain retinal pathologies such as senile macular degeneration and retinitis pigmentosa.

The sensitivity of the retina to blue light may have more profound effects for man-made optical sources than for sunlight. The trend towards ever brighter sources in the blue end of the spectrum—high pressure Hg and xenon arc lamps, and the 'natural' lighting sources in current use—are examples. The ocular hazards associated with lasers may require re-evaluation. Arc welding is another example where the eyes may need special protection from blue light. Children undergoing phototherapy for bilirubinemia should have their eyes protected and the growing population of elderly people with aphakic eyes will need

protection from the short wavelengths in the visible and near ultraviolet spectrum.

The basic photochemical mechanisms underlying the sensitivity of the retina to short wavelengths of visible light are unknown. Our preliminary results indicate that the lesion is located in the outer segment of the photoreceptor and perhaps in the pigment epithelium. We have not as yet been able to distinguish between rod and cone damage and do not know to what extent the photopigments are involved. Most previously reported long term photic damage results from exposure to bright environmental lighting over periods of days or weeks, whereas our exposures extend from 1–1,000 s and are confined to an extremely small and finite portion of the retina. Anderson *et al.*¹⁵ have reported similar sensitivity to blue light in albino rats in environmental lighting conditions. A number of mechanisms to explain long term photic damage to the retina have been proposed. These include photo-oxidative membrane damage, toxic products in the outer retina, metabolic disorders from extended overbleach, and so on. In our opinion the continued increase in sensitivity with decreasing wavelength indicates that additional molecular systems become vulnerable to electronic excitation and photic damage as the energy per photon increases. This is to be expected and we believe that several photochemical mechanisms are probably responsible for the observed damage. We do not expect the action spectrum to peak as the near ultraviolet is approached.

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WILLIAM T. HAM, JR
HAROLD A. MUELLER

Department of Biophysics,
Virginia Commonwealth University,
Richmond, Virginia 23298

DAVID H. SLINNEY

US Army Environmental Hygiene Agency,
Edgewood Arsenal, Maryland 21010

Received November 10, 1975; accepted January 14, 1976.

- ¹ Noell, W. K., Walker, V. S., Kang, B. S., and Berman, S., *Invest. Ophthalmol.*, **5**, 450-473 (1966).
- ² Kuwabara, T., and Gorn, R. A., *Archs Ophthalmol.*, **79**, 69-78 (1968).
- ³ Ts'o, M. O. M., Fine, B. S., and Zimmerman, L. E., *Am. J. Ophthalmol.*, **73**, 686-699 (1972).
- ⁴ Lawwill, T., *Invest. Ophthalmol.*, **12**, 45-51 (1973).
- ⁵ Harwerth, R. S., and Sperling, H. G., *Science*, **174**, 520-523 (1971).
- ⁶ O'Steen, W. K., Shear, C. R., and Anderson, K. V., *Am. J. Anat.*, **134**, 5-22 (1972).
- ⁷ Vassiliadis, A., Report SA-3314-17 prepared for Int. Bus. Machines Corp., PO Box 12195, Research Triangle Park, North Carolina 27709 (1975).
- ⁸ Gibbons, W. D., and Allen R. G., Report SAM-TR-75-11, 1-11, USAF, School of Aerospace Medicine, Brooks AFB, Texas 78235 (1975).
- ⁹ Ham, W. T., Jr, Mueller, H. A., Williams, R. C., and Geeraets, W. J., *Appl. Opt.*, **12**, 2122-2129 (1973).
- ¹⁰ Ts'o, M. O. M., and LaPiana, F. G., *Trans. Am. Acad. Ophthalmol. Otolaryn.*, **78**, 677-678 (1974).
- ¹¹ White, T. J., Mainster, M. A., Wilson, P. W., and Tips, J. H., *Bull. Math. Biophys.*, **33**, 1-17 (1971).
- ¹² Clarke, A. M., Geeraets, W. J., and Ham, W. T., Jr, *Appl. Opt.*, **8**, 1051-1054 (1969).
- ¹³ Smith, H. E., *US Navy med. Bull.*, **42**, 675-680 (1944).
- ¹⁴ Marlor, R. L., Blais, B. R., Preston, F. R., and Boyden, D. G., *Invest. Ophthalmol.*, **12**, 5-16 (1973).
- ¹⁵ Anderson, K. V., Coyle, F. P., and O'Steen, W. K., *Expl Neurol.*, **35**, 233-238 (1972).

Visual adaptation produced by gratings with no brightness contrast

A STRIPED grating is more difficult to see after prolonged viewing of a similar grating^{1,2}. Similarly, the contrast threshold is elevated when a test grating is briefly presented superimposed on a continuously viewed grating^{3,4}. Here we show that the threshold is raised by gratings that have no brightness difference between alternate stripes, but only a hue difference. We measured luminance modulation thresholds for seeing a yellow grating superimposed on a background of interdigitated red and green stripes. We varied the relative luminances of the red and green stripes while holding the sum of their luminances constant. Thus we could be certain that for some luminance ratio, the red and green stripes must have had the same subjective or effective brightness for the observer. Nevertheless, we found that for all ratios of red-to-green luminance the contrast threshold for the test grating was higher than that obtained in several control conditions (including backgrounds with the same colours and configuration but perpendicular to the test grating, and uniform backgrounds with the same mean spectral composition).

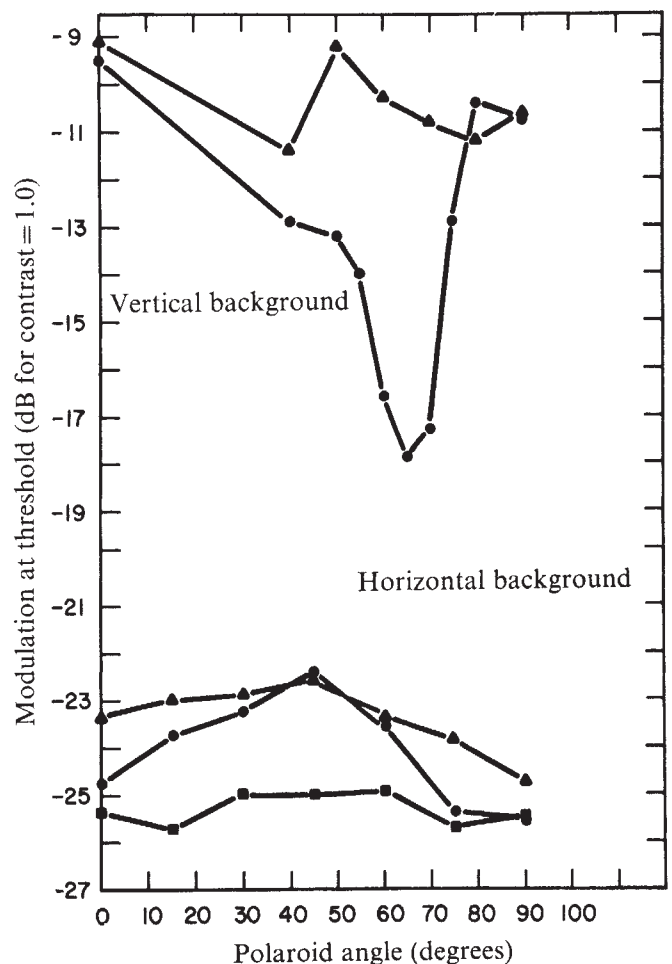
Yellow test gratings were produced by conventional techniques on an oscilloscope with P31 phosphor, viewed at a distance of 3 m through an Ilford 201 filter. The test field was a 13 cycles per degree sine grating, 2° square. The background gratings, also 13 cycles per degree, were produced by two projectors whose beams fell on a ground glass screen after being merged by a half-reflecting pellicle. Each projector cast an image of a square-wave grating on the screen. An Ilford 624 (bright spectrum green) filter was placed in the beam of one projector, and an Ilford 205 (narrow cut tricolour red) filter in the beam of the other. Polaroid filters oriented perpendicularly with respect to each other were also interposed in the separate beams. The relative intensities of the red and green components could then be varied by rotating a Polaroid interposed in the common beam. The positions of the red and green gratings could be adjusted so as to superimpose the red and green stripes or to interdigitate them. When red and green were superimposed, the grating varied in appearance from red-and-black through yellow-and-black to green-and-black as the Polaroid in the common path was rotated. When red and green were interdigitated, the appearance varied from red-black through red-green to black-green. The screen was viewed from the side opposite the projectors at a distance of 3 m, and was seen through a pellicle that merged its image with that of the test grating. A dove prism close to this pellicle, but only in the path to the background screen, controlled the orientation of the background grating. Various adjustments allowed for the precise control of orientation, phase, and magnification of test and background gratings. The mean luminances over the area at the observer's eye were 6.2 and

3.9 cd m⁻² for the red and green components at their respective maximum settings and 6.2 cd m⁻² for the test field.

Contrast thresholds were measured by a two-staircase up-and-down procedure. When the observer pressed a key the test screen was changed from homogeneous to striped for 100 ms. If he saw the test grating, its contrast was reduced by 1 dB (about 12%) on the next trial on that staircase. If he did not see it, it was raised by 1 dB. Random selection of the staircase operative on each trial reduced the observer's ability to predict the changes in contrast from trial to trial. All of the results are contrast thresholds for detection of a yellow vertical sine grating; therefore any differences in thresholds are due to the differences in the backgrounds on which the test grating was presented.

Figure 1 plots the contrast thresholds for the test grating on various backgrounds. The x axis represents the relative luminances of the red and the green background stripes, as determined by the setting of the variable Polaroid. Thresholds were nearly independent of Polaroid angle for all background conditions except vertical interdigitated red-green gratings. With interdigitated vertical gratings, however, the threshold was found to vary with relative luminance of the red and green stripes, reaching a minimum at a Polaroid angle of about 65°. Even at this point, though, the threshold was elevated substantially—5 dB higher than in any of the three control

Fig. 1 Contrast thresholds for a vertical yellow test grating (13 cycles per degree) presented briefly against various backgrounds: interdigitated red and green stripes, superimposed red and green stripes, uniform yellow field. Relative luminance of red and green components was varied by rotating a Polaroid filter in the dual optical system. For interdigitated backgrounds Polaroid settings of 0° produced red-black and 90° black-green gratings respectively. All data are from the same observer. Mean s. d. of observations, 0.94 dB. ●, Interdigitated stripes; ▲, superimposed stripes; ■, uniform background.



conditions (horizontal interdigitated, horizontal superimposed stripes, or uniform backgrounds of the same mean luminance and mean spectral composition as the striped fields). Essentially the same result has been obtained in several repetitions of this experiment using other grating frequencies (3 and 6 cycles per degree) and a second observer. We have always found some threshold elevation (at least 5 dB) with any luminance ratio of the red-green stripes. Thus it seems reasonable to conclude that threshold elevation can occur without luminous contrast in the adapting stimulus.

In this experiment the more intense stripes of the yellow test grating were aligned with the green stripes of the interdigitated backgrounds. An auxiliary experiment tested whether the relative position of background and test gratings had any effect on the contrast thresholds. Phase was varied in 30° steps from the condition where the more intense yellow steps were superimposed on the green background stripes to that where they were superimposed on the red background stripes. There was no systematic effect of phase. Threshold was 6 dB higher than the control condition with a maximum deviation of any point of 1 dB from the mean. Thus the variation of contrast thresholds with Polaroid angle (Fig. 1) cannot be attributed to phase effects.

Although thresholds are never as low on red-green vertical backgrounds as they are on the various control backgrounds, they do reach a minimum for an intermediate setting of the Polaroid, that is, when the luminance contrast is small or non-existent. As shown in Fig. 1, this does not happen when the red and green stripes are in phase.

Two interpretations of the minimum in the curve with red-green interdigitated stripes are plausible. On the one hand, adopting a form of opponent-colour theory, it may be supposed that pattern information is carried by both luminance and chromatic channels. When the Polaroids were set at their extreme positions, producing red-black or green-black gratings, both classes of channels were activated, but in the middle range only the chromatic channels received inputs, thus resulting in less elevation of the threshold. Alternatively, it might be held that the sum of the contrasts in the primary cone mechanisms determines the degree of threshold elevation. Since the red light used stimulates the green cone mechanism (π_4) as well as the red cone mechanism (π_5) and the green light π_5 as well as π_4 , the sum of the contrast in these mechanisms is greatest at the extreme settings of the Polaroid and reaches a minimum for some intermediate setting. It is not possible with the available data to choose between these alternative hypotheses.

From a simple, subjective point of view, it is perhaps not surprising that equally bright red and green stripes elevate the threshold for seeing gratings, since they are visible as gratings. Perhaps the threshold elevation is simply related to the visibility of the adapting pattern.

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JOHN KRAUSKOPF

*The Physiological Laboratory,
Cambridge University,
and Bell Laboratories,
Murray Hill, New Jersey 07974*

IAN CAMPBELL

*The Physiological Laboratory,
Cambridge University,
Downing Street,
Cambridge, UK*

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Dissociation of response to injected gonadotropin between the Graafian follicle and oocyte in pigs

THE process of ovulation in mammals is induced by a surge of gonadotropic hormone(s) that precipitates a series of changes in the cellular layers of the Graafian follicle. The changes in the somatic cells are extensive, ranging from enzymatic transformation in the biosynthetic pathway for steroid hormones^{1,2} to morphological alterations in the membrana granulosa. In contrast, the only conspicuous modification in the germ cell is completion of the first meiotic division with extrusion of a polar body and formation of the second metaphase plate. The meiotic steps from diakinesis to first polar body formation are known to be programmed with considerable accuracy^{3,4} by the endogenous surge of luteinising hormone (LH), but a similar sequence of nuclear changes can also be initiated by injection of a preparation rich in LH activity, such as human chorionic gonadotropin (HCG)⁵⁻⁷. Although the changes in the somatic cells and oocyte within the mature follicle usually occur in concert, we describe here circumstances in which the process of ovulation is dissociated from nuclear maturation of the germ cell, resulting in the shedding of a primary oocyte.

Mature Large White gilts with 20- or 21-d oestrous cycles were assigned to one of four groups and given a single intramuscular injection of 500 or 1,000 IU of HCG (Organon) in saline on day 17, 18, 19 or 20 of the cycle. Ovulation occurs 40-42 h after such an injection given during pro-oestrus^{8,9}. Animals were tested immediately before injection with a mature boar to ensure that they were not in oestrus, a procedure continued twice daily until slaughter. Between 24 and 30 h after injection of HCG, laparotomy was performed under Nembutal-halothane anaesthesia and 5 ml of semen containing $1.99-2.74 \times 10^8$ spermatozoa per ml was instilled into each uterine horn. Animals were slaughtered 44-72 h after receiving HCG, and ova were flushed from the Fallopian tubes with Eagle's medium. These were fixed in acetic alcohol, stained with orcein and examined by phase contrast microscopy.

In 15 control animals, the steroid hormone environment of the oocyte was estimated in mid-follicular phase and shortly before ovulation. Follicular fluid was obtained at laparotomy during occlusion of the ovarian blood supply or immediately on ovariectomy, by aspiration into 1-ml plastic syringes through a 26-gauge needle. Follicular fluid was stored at -20 °C until it could be analysed for oestradiol by radioimmunoassay; the method was essentially that of Hotchkiss *et al.*¹⁰ but used an antiserum raised in sheep against 6-oxoestradiol coupled through a carboxymethyl-oxime bridge to bovine serum albumin. Progesterone was estimated by radioimmunoassay¹¹ using an antiserum raised in sheep against 11 α -hydroxyprogesterone coupled through a succinate bridge to bovine serum albumin. Follicular fluids from animals injected with HCG were also examined by radioimmunoassay for LH activity.

Table 1 summarises the number of animals injected; the responses can be considered in terms of the maturation of several systems. Display of behavioural oestrus was exceptional after HCG injection on day 17 or 18, but it was a predictable sequel to treatment in the later part of the follicular phase. Likewise, after injection on day 17, the ovarian response to HCG was usually superovulation, whereas the mean ovulation rates after an injection on day 18, 19 or 20 did not differ significantly from those arising spontaneously in comparable animals.

The nuclear maturity of oocytes recovered from the Fallopian tubes was also related to the time of HCG injection.

¹ Gilinsky, A., *Opt. Soc. Am.*, **58**, 13 (1968).

² Blakemore, C., and Campbell, F. W., *J. Physiol., Lond.*, **203**, 237 (1969).

³ Campbell, F. W., and Kulikowski, J. J., *J. Physiol., Lond.*, **187**, 437 (1966).

⁴ Harris, C. S., and Krauskopf, J., *Bull. Psychonomic Soc.*, **2**, 325 (1973).

Table 1 Responses to HCG

Day of oestrous cycle* when HCG injected	System under consideration	Behavioural oestrus		Ovarian response		Maturity of oocyte nucleus	Block to polyspermy	Availability of vitelline factors (MPGF)†
	No. of animals injected	Animals showing oestrus		Recent ovulations		Primary oocytes (%)	Polyspermic eggs (%)	Eggs containing transformed sperm heads (%)
		No.	(%)	Total no.	Mean per animal			
17	7	0	0	130	18.6	18.2	91.7	< 10
18	9	1	11.1	112	12.4	17.4	38.0	
19	45	39	86.7	581	12.9	2.1	2.4	
20	27	25	92.6	308	11.4	1.4	2.8	> 90

Figures show results obtained from 88 post-puberal pigs illustrating five aspects of the response to a single intramuscular injection of HCG given at various stages of the follicular phase of the oestrous cycle. These results can be considered in terms of a series of biological systems which undergo maturation during the latter part of the oestrous cycle.

*Only animals with oestrous cycles lasting 20 or 21 d were assigned to this experiment.

[†]MPGF, Male pronucleus growth factor¹⁴.

tion: most oocytes recovered after day 17 treatment possessed a dictyate nucleus in which the chromatin was condensed around a single large nucleolus. Such primary oocytes were tightly encompassed by cumulus cells. By contrast, after HCG injection on day 19 or 20, recovery of primary oocytes was rare, only 14 being observed among 785 ova examined from these groups.

Maturation of the mechanism underlying the block to polyspermy was also inferred to be taking place during the follicular phase, since 92% of the oocytes recovered after treatment on day 17 exhibited polyspermy compared with 2–3% after treatment on day 19 or 20 (Table 1). Moreover, the extent of the polyspermic condition in individual primary oocytes was frequently massive, with as many as 60–80 unswollen sperm heads in the egg cytoplasm and still more spermatozoa in the perivitelline space. Other systems in the egg cytoplasm were evidently undergoing maturation during the follicular phase, as judged by the failure of sperm heads within the vitellus of primary oocytes to decondense and form pronuclei. This metamorphosis is thought to be promoted by interaction between the sperm head and egg cytoplasm^{12–14}, and is an essential step in the formation of a spherical pronucleus.

Assay of samples of follicular fluid revealed a marked variation in the concentration of oestrogens between follicles from the same animal, ranging from 15–210 ng ml⁻¹ on day 17 of the cycle to 108–750 ng ml⁻¹ on day 20, shortly before the onset of behavioural oestrus. There was also considerable variation between animals in the oestrogen content of follicular fluid aspirated on the same day of the cycle. Nevertheless, an overall trend of an increasing concentration of oestrogens in follicular fluid could be plotted from day 17 (mean 75.3 ± 8.7 ng ml⁻¹) to day 20 (263.8 ± 54.5 ng ml⁻¹), these values decreasing only after the onset of oestrus or HCG injection. Twenty-four hours after the latter event(s), the concentration of follicular oestrogens was frequently reduced to 6–40 ng ml⁻¹ (mean 20.7 ± 5.1 ng ml⁻¹), whereas progesterone titres were as high as 3,400–6,400 ng ml⁻¹. The corresponding values for the concentration of oestrogens in peripheral plasma increased from 42 pg ml⁻¹ on day 17 to 190 pg ml⁻¹ on day 20, decreasing to 25–30 pg ml⁻¹ 24 h after the onset of oestrus.

These observations show that nuclear and cytoplasmic maturation of the oocyte are not essential concomitants of the final maturation of the Graafian follicle, at least when ovulation is induced by HCG in the mid-follicular phase of the oestrous cycle. The dissociation of the response of the somatic cells from that of the germ cell obtained in such a situation makes it reasonable to question any direct involvement of the oocyte in regulating the destiny of the follicle and its membrana granulosa¹⁵, as suggested by studies of pigs and rabbits involving ovariectomy^{16,17} and of rats involving cultured oocytes and granulosa cells¹⁸.

The basic question arising from this work is the manner

in which the oocyte normally responds to gonadotropic stimulation, and the nature of the failure to resume meiotic maturation in conditions of precocious ovulation induced by HCG. Previous work has demonstrated receptors for LH on porcine granulosa cells¹⁹, and also that HCG can bind to these cells^{19,20}. Accordingly, we propose that the increasing titres of intrafollicular oestrogens that arise during the latter part of the oestrous cycle mediate in the response of the oocyte to the gonadotropin stimulus, in part at least by increasing the binding sites available for LH or HCG or the granulosa cells; these sites have been reported to be similar²¹. Failure of the oocyte to respond after day 17 HCG injection is not due to a lack of gonadotropic hormone reaching the granulosa cells, since high levels of LH activity have been detected in follicular fluid within 4 h of injection of HCG (unpublished results of W. R. Carr and R.H.F.H.). In a broader biological context, the proposed involvement of oestrogens within the ripening follicle seems fundamental, since oocyte maturation would normally occur only in circumstances in which the animal was exhibiting behavioural oestrus, and where the reproductive tract was tonic and oedematous. In other words, an integrating gonadal hormone is needed during the late follicular phase to coordinate changes within the follicle, the reproductive tract and the hypothalamo-pituitary axis.

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R. H. F. HUNTER

School of Agriculture,
University of Edinburgh,
West Mains Road,
Edinburgh EH9 3JG, UK

B. COOK

Department of Steroid Biochemistry,
University of Glasgow,
Glasgow, UK

T. G. BAKER

Department of Obstetrics and Gynaecology,
University of Edinburgh, UK

Received December 12, 1975; accepted January 28, 1976.

- Short, R. V., *Rec. Prog. Hormone Res.*, **20**, 303–333 (1964).
- Lindner, H. R., *et al.*, *Rec. Prog. Hormone Res.*, **30**, 79–127 (1974).
- Pincus, G., and Enzmann, E. V., *J. exp. Med.*, **62**, 665–675 (1935).
- Spalding, J. F., Berry, R. O., and Moffit, J. G., *J. Anim. Sci.*, **14**, 609–620 (1955).
- Edwards, R. G., and Gates, A. H., *J. Endocr.*, **18**, 292–304 (1959).
- Dziuk, P. J., *Anat. Rec.*, **153**, 211–224 (1965).
- Hunter, R. H. F., and Polge, C., *J. Reprod. Fert.*, **12**, 525–531 (1966).
- Dziuk, P. J., Polge, C., and Rowson, L. E., *J. Anim. Sci.*, **23**, 37–42 (1964).
- Hunter, R. H. F., *Vet. Rec.*, **81**, 21–23 (1967).
- Hotchkiss, J., Atkinson, L. E., and Knobil, E., *Endocrinology*, **89**, 177–183 (1971).
- Thornycroft, I. H., and Stone, S. C., *Contraception*, **5**, 129–145 (1972).
- Austin, C. R., *The Mammalian Egg*, 47–48 (Blackwell, Oxford, 1961).
- Hunter, R. H. F., *J. exp. Zool.*, **165**, 451–460 (1967).
- Thibault, C., and Gérard, M., *C. r. hebdom. Séanc. Acad. Sci., Paris*, **270**, 2025–2026 (1970).
- Hunter, R. H. F., and Baker, T. G., *J. Reprod. Fert.*, **43**, 193–196 (1975).
- El-Fouly, M. A., Cook, B., and Nalbandov, A. V., *Anat. Rec.*, **166**, 302, Abstr. (1970).
- El-Fouly, M. A., Cook, B., Nekola, M., and Nalbandov, A. V., *Endocrinology*, **87**, 288–293 (1970).
- Nekola, M. V., and Nalbandov, A. V., *Biol. Reprod.*, **4**, 154–160 (1971).

¹⁹ Channing, C. P., and Kammerman, S., *Endocrinology*, **92**, 531-540 (1973).

²⁰ Schomberg, D. W., and Tyrey, L., *Biol. Reprod.*, **7**, 127, Abstr. (1972).

²¹ Kammerman, S., Canfield, R. E., Kolena, J., and Channing, C. P., *Endocrinology*, **91**, 65-74 (1972).

Bilateral electroencephalographic response and unilateral tolerance to unilateral intracerebral morphine injections

DRUG tolerance is characterised by a loss of sensitivity to the pharmacological properties of a drug as a result of repeated exposure. Because it can be produced by both intravenous and intracerebral injection of opiates or barbiturates¹⁻³, functional alteration of neural cells probably contributes to its development. The identification of mechanisms underlying the development of tolerance to morphine in the central nervous system is complicated by secondary drug-induced changes in bioelectrical and metabolic activity. This can be circumvented by the use of a mirror-focus preparation where comparison of the drug-treated region in one hemisphere can be made with the morphological homologue in the contralateral hemisphere. For example, Morrell⁴ induced bilateral epilepsy after unilateral treatment with an irritant that causes the epileptic discharge pattern to be propagated, through the commissural projections, to the mirror-focus cells in the opposite hemisphere. Since the mirror-focus cells are never in direct contact with the epileptogenic agents, any alteration in their metabolism is due solely to epilepsy. Similarly, seizures generated by electrical stimulation of one amygdala propagate to the contralateral amygdala^{4,5}. Since the anterior amygdala of the rat has a high concentration of opiate receptors⁶, we have used it to investigate the effects of direct unilateral injection of morphine sulphate. We found that injections of opiates such as morphine and levorphanol produce seizures at the injection site that propagate to the mirror-focus in the contralateral hemisphere. Tolerance to opiates develops at the injection site but the mirror-focus retains normal sensitivity to opiates in spite of the shared epileptiform activity.

12 MINUTES AFTER DEXTROPHAN (10.8 μ g IN 0.9 μ l) R. AMYG

L. AMYG

R. AMYG

200 μ V
1

CORTEX

11 MINUTES AFTER MORPHINE (9 μ g IN 0.9 μ l) R. AMYG

L. AMYG

R. AMYG

200 μ V
1

CORTEX

Fig. 2 Comparison of the effects of morphine and dextrophan injected at the same site. Spike and wave pattern is evident after morphine. Dextrophan has no effect. Cortical tracings are unaffected by amygdala injections.

Twenty-two Sprague-Dawley rats were anaesthetised with sodium pentobarbital and bilateral cannula-recording electrode assemblies were implanted in the anterior amygdala. The permanently implanted cannulae serve as a guide for smaller fluid delivery cannulae and as one pole of the bipolar recording assembly that monitors changes in electrical activity at the injection site. Injections of opiates

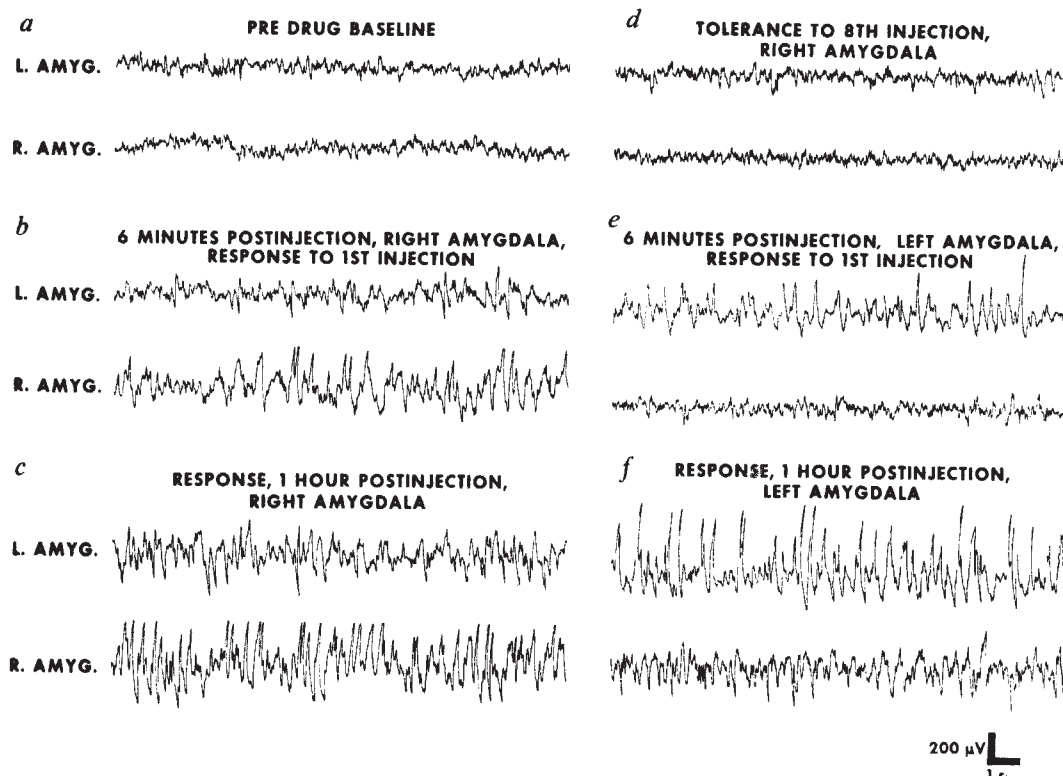


Fig. 1 Lateralisation of morphine tolerance to the right amygdala as a result of eight consecutive microinjections (2.7 μ g in 0.9 μ l) of morphine. In spite of the shared epileptiform activity of the mirror-focus in the left hemisphere, tolerance to morphine is restricted to the original injection site.

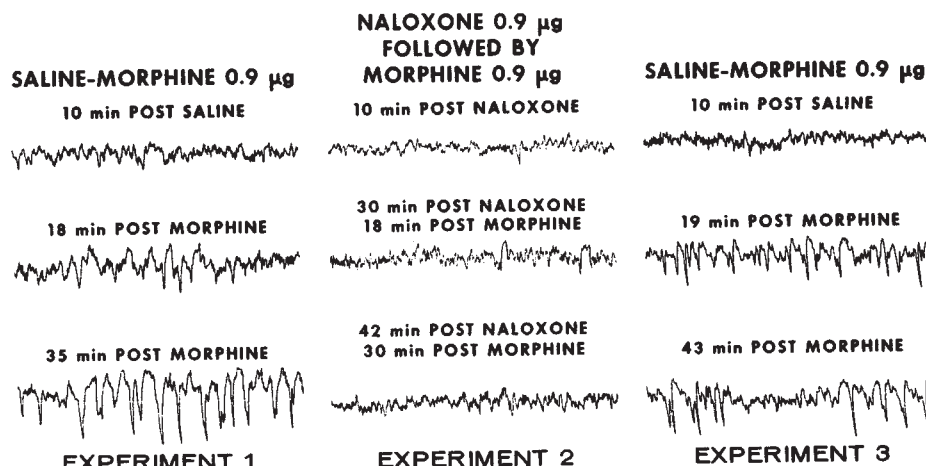


Fig. 3 Antagonism of morphine-induced changes in amygdala electroencephalographic activity by preinjection of an equal dose of naloxone. Experiments 1 and 3 were carried out 2 d before and after naloxone challenge.

and control injections of saline were delivered at a rate of $0.03 \mu\text{l s}^{-1}$ during 30 s.

After injecting morphine sulphate (2.7 or $9.0 \mu\text{g}$) unilaterally into the anterior amygdala, we observed a spike and wave pattern at the injection site in 17 of 22 animals (Fig. 1a and b). The pattern appeared at the contralateral amygdala 15–30 min after injection and persisted for 3–4 h (Fig. 1c) each day. Injections of saline had no effect at either recording site. After repeated daily injections, tolerance manifested by a diminished bioelectric response developed at the injection site (Fig. 1d).

In spite of the daily appearance of sustained epileptiform patterns of the mirror-focus in the opposite hemisphere, tolerance was not transferred to the opposite hemisphere. When a similar dose of morphine was administered directly to the previously untreated amygdala, sensitivity to the drug was normal at the mirror-focus (Fig. 1e). Furthermore, kindled spike and wave patterns were seen at the original injection site (Fig. 1f). Levorphanol ($3.0 \mu\text{g}$) caused a similar response when injected into the amygdala. Furthermore, cross tolerance between the two opiates has been observed (our unpublished observations). Dextrorphan ($10.8 \mu\text{g}$), however, elicited no apparent electroencephalographic response (Fig. 2).

Several lines of evidence indicate that the electroencephalographic response of the amygdala elicited by acute morphine administration, and the eventual disappearance of this response with chronic morphine treatment is not a toxic or nonspecific effect.

Cannula location is critical in the production of this response. Histological examination showed that cannulae placed bilaterally over the anterior amygdala gave bilateral opiate responses. If the tips of the cannulae were in the ventrolateral striatum or deep in the pyriform cortex, no morphine response was seen. The localisation of this response to the anterior amygdala is consistent with reports of high concentrations of opiate receptors in this region.

Although chronic morphine administration led to diminution of the response of the amygdala, an increase in the dose resulted in the reappearance of the response seen with acute doses. The loss of sensitivity seen after repeated injections of morphine could not have been the result of consequent tissue distortion, because a week of daily control injections of an equal volume of saline had no effect on morphine sensitivity of the amygdala ($N=3$). The response to the opiates is stereospecific—levorphanol but not dextrorphan mimics the action of morphine. Naloxone has no apparent effect when administered by itself but blocks the effect of subsequent morphine administration. Pretreatment with an equal dose of naloxone (10 min before morphine) completely blocked the morphine response (Fig. 3) seen on control days

before (experiment 1) and after (experiment 3) the naloxone test when sensitivity to morphine was normal ($N=4$).

Evoked potential thresholds (produced by electrical stimulation of the contralateral amygdala) were unchanged in spite of the appearance of drug-induced alterations in electroencephalogram patterns at the injection site. Recordings always returned to baseline after drug administration.

These findings show that unilateral tolerance can be produced by unilateral intracerebral microinjection of morphine sulphate into the amygdala. Furthermore, the morphine-induced epileptiform patterns shared by both amygdala do not contribute to the development of drug tolerance.

Although tolerance to direct intracerebral injections of opiates and barbiturates has already been demonstrated^{1,3}, this is the first demonstration of lateralisation of opiate tolerance. We plan to relate this unilateral alteration in drug sensitivity to an ipsilateral alteration in biochemical activity that is not seen at the contralateral site in the brain in spite of identical morphology and shared epileptiform experience.

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H. TEITELBAUM
J. BLOSSER
G. CATRAVAS

Departments of Behavioral Sciences and Neurobiology,
Armed Forces Radiobiology Research Institute,
Bethesda, Maryland 20014

Received August 18; accepted October 7, 1975.

- 1 Lotti, V. J., Lomax, P., and George, R., *Int. J. Neuropharmac.*, **5**, 35–42 (1966).
- 2 Jaffe, J., and Sharpless, S. K., *Pharmacologist*, **5**, 249 (1963).
- 3 Mycek, M. J., and Brezenoff, H. E., *Fedn Proc.*, **33**, 527 (1974).
- 4 Morrell, F., in *Chemical Modulation of Brain Function* (edit. by Sabelli, H. C.) (Raven, New York, 1973).
- 5 Goddard, G. V., *Nature*, **214**, 1020–1021 (1967).
- 6 Kuhar, M. J., Pert, C. B., and Snyder, S. H., *Nature*, **245**, 447–450 (1973).

Changes in sensitivity of morphine-induced circling behaviour after chronic treatment and persistence after withdrawal in rats

BEHAVIOURAL effects of morphine may be mediated, at least in part, by functional changes in the dopaminergic nigrostriatal system¹. Thus, for example, acute administration of morphine increases the rate of dopamine turnover in the striatum (for examples refs 2–4), and nigrostriatal lesions block symptoms of morphine withdrawal^{5–7}. Spontaneous turning in circles after drug treatment in rats has been reported

to be an index of an intrinsic asymmetry between left and right nigrostriatal pathways⁸⁻¹². We now report that both acute morphine administration and cessation of chronic morphine administration induce this rotation, which persists for at least 2 months after withdrawal. The data suggest that morphine induces the synthesis of an endogenous morphine-like substance¹³⁻¹⁵ which persists long after morphine is eliminated.

The subjects were naive female Sprague-Dawley rats weighing approximately 260 g. For measuring rotation, rats were placed individually in a completely automated rotometer¹⁶ which differentiates between complete 360° rotations and incomplete oscillatory turns. After habituation for 15 min each rat was injected intraperitoneally with morphine sulphate or saline. Rotations were recorded on a printout counter during the 60 min after injection. Rotations to the left or right were totalled separately and the net positive rotational difference (that is rotations in the dominant direction minus rotations in the opposite direction) was determined for each rat.

In the preliminary acute dose-response studies, doses from 0.25 to 20.0 mg kg⁻¹ were tested (12 rats per dose). Morphine induced significant ($P < 0.05$, t tests) rotation at 2.5, 5 and 10 mg kg⁻¹; with larger doses rats became increasingly cataleptic. Mean net rotations per hour $\pm$ s.e. for 0 (saline), 0.25, 1.0, 2.5, 5.0, 10.0 and 20.0 mg kg⁻¹ were, respectively: 2.2 ± 0.6 , 5.8 ± 1.4 , 5.7 ± 1.6 , 7.2 ± 2.3 , 12.7 ± 3.9 , 10.0 ± 2.6 and 4.8 ± 1.4 . As with other drugs^{8,11}, the rotation induced by morphine was consistent in direction and magnitude; that is, when retested at weekly intervals with 5.0 mg kg⁻¹, some rats consistently rotated to the right and others to the left, and the numbers of net rotations were significantly correlated ($r = +0.55$ to 0.61 , $P < 0.01$, t test) from week to week. The rotation cannot be attributed to hyperactivity. If an animal were only hyperactive there might be more turns, but there is no reason *per se* why such turns should be unevenly distributed, in any consistent way, to the left or right. It seems that net rotations are inversely correlated with locomotor activity¹⁷.

In the experiments involving chronic administration, 16 rats were initially (day 1) tested for rotation following an injection of morphine (5.0 mg kg⁻¹). A day later, silicone reservoirs were implanted subcutaneously in all rats as described by Goode¹⁸. On the next day, chronic morphine administration was begun for 3 d (days 3, 4 and 5). Each morning the reservoirs were rinsed with physiological saline and refilled with 0.5 ml of morphine sulphate solution in a saline vehicle (50 mg ml⁻¹). On the withdrawal day (day 6), the reservoirs were rinsed and filled only with saline. Immediately after removal of morphine from the reservoirs, each rat was placed in a rotometer and, 15 min later, injected with either morphine (5.0 mg kg⁻¹; group 1, $N = 8$) or saline (group 2, $N = 8$). Each rat was tested again for rotation after receiving the same

dose of morphine or saline 1, 3, 7, 15, 30 and 60 d after withdrawal of chronic morphine (days 7, 9, 13, 21, 36 and 66 in Table 1). The body weight of each rat was recorded before the first injection of morphine (day 1) and on every day thereafter that rats were handled.

The results are shown in Table 1. After withdrawal of morphine on day 6, the rats in group 1 were somewhat tolerant to the rotatory effect of morphine and the rats in group 2 did not rotate after receiving saline. Mean net rotations in group 2 were not significantly different ($P > 0.1$, t test) from an acute effect of saline in naive rats ($N = 12$) nor from an effect of saline in rats implanted with reservoirs filled only with saline ($N = 4$). By 3 d after withdrawal (day 9), the rats injected with morphine were obviously more sensitive to the rotatory effect of morphine and the rats injected with saline rotated as much as they had previously in response to their first (day 1) injections of morphine. Both of these latter effects were greater 7 d after withdrawal (day 16) and remained stable thereafter on all subsequent testing days. In contrast, body weight changes showed a typical early withdrawal pattern^{7,19}—a decrease in body weight 1–3 d after withdrawal followed by a return to pre-withdrawal weights within 7–10 d. The small numbers of net rotations (approximately two per hour) induced by saline in naive rats were not consistent in direction and did not increase in magnitude when rats were retested at weekly intervals for up to 2 months. Since saline does not normally induce rotation, withdrawal-induced rotation seems to be a genuine manifestation of a protracted abstinence syndrome²⁰.

Correlations of day 1 morphine-induced rotation with withdrawal-induced rotation, as well as with morphine-induced rotation after withdrawal, suggest that chronic administration of morphine induces the synthesis of a morphine-like substance¹³⁻¹⁵ responsible for the prolonged rotatory effects observed. That is, the direction of rotation of each rat was consistent across all tests, and saline and morphine data from 3 to 60 d after withdrawal were quantitatively correlated ($r = +0.64$ to 0.71 , $P < 0.01$, t test) with day 1 data; the more a rat rotated in response to an acute injection of morphine, the more it also rotated during the withdrawal tests. In previous studies²¹ with other drugs, we have generally found that although rotation is always consistent from week to week, when rats are retested with the same drug, there are no positive and quantitative correlations between drugs (for example, rats may rotate in opposite directions with (+)-amphetamine and apomorphine)—such differences probably reflect different ways in which drugs can effect the intrinsic cerebral asymmetry²¹. The correlation between acute morphine-induced rotation and withdrawal-induced rotation suggests, therefore, that long after morphine is eliminated an endogenous substance persists which has at least one pharmacological action resem-

Table 1 Rotation and body weight after chronic morphine administration

Group 1				Group 2		
Day	Treatment	Rotation	Body weight	Treatment	Rotation	Body weight
1	M*	100†	100‡	M	100†	100‡
2	Surgery	—	—	Surgery	—	—
3–5	Chronic M	—	—	Chronic M	—	—
6	Withdraw M;M	71.9 $\pm$ 22.2	105.5 $\pm$ 1.5	Withdraw M;S	31.9 $\pm$ 15.3	104.7 $\pm$ 1.3
7	—	—	—	S	66.7 $\pm$ 12.5	99.6 $\pm$ 1.1
9	M	180.7 $\pm$ 35.4	97.8 $\pm$ 2.0	S	101.4 $\pm$ 29.2§	99.6 $\pm$ 1.3
13	M	249.0 $\pm$ 42.6	101.4 $\pm$ 1.9	S	220.8 $\pm$ 36.5	106.7 $\pm$ 1.0¶
21	M	141.7 $\pm$ 41.6	106.4 $\pm$ 2.1¶	S	158.3 $\pm$ 47.4	112.8 $\pm$ 1.5
36	M	231.8 $\pm$ 37.1	116.8 $\pm$ 2.0	S	240.3 $\pm$ 41.6	122.3 $\pm$ 2.0
66	M	172.2 $\pm$ 33.8	131.3 $\pm$ 2.1	S	170.8 $\pm$ 31.7	131.8 $\pm$ 2.1

Rotation and body weight are expressed as mean percentage of day 1 $\pm$ s.e.

*M alone refers to morphine sulphate, 5.0 mg kg⁻¹, intraperitoneal; chronic M or withdraw M refers to morphine administered through implanted (day 2) silicone reservoirs; S refers to saline, 0.1 ml, intraperitoneal.

†Mean net rotations per hour $\pm$ s.e. for all rats on day 1 were 11.2 ± 4.8 .

‡Mean body weight (g) $\pm$ s.e. for all rats on day 1 was 255.6 ± 3.9 .

§For all data points except this one, rotation was significantly different ($P < 0.05$ – 0.1 , t tests) from day 1.

¶For all data points except these, body weight was significantly different ($P < 0.05$ – 0.1 , t tests) from day 6 (pre-withdrawal).

bling that of morphine. This interpretation is consistent with the evidence that rotation is indicative of dopaminergic striatal function⁸⁻¹², that morphine is known to affect dopaminergic mechanisms in the striatum¹ and that the highest concentrations of opiate receptors²² and endogenous morphine-like substances¹³⁻¹⁵ are found in the striatum. Although there are other reports²³⁻²⁵ of long term after effects of chronic morphine administration, the study reported here provides the first demonstration of an after effect resembling an acute effect of morphine.

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STANLEY D. GLICK
JOHN M. MORIHISA

Department of Pharmacology,
Mount Sinai School of Medicine,
Fifth Avenue and 100th Street,
New York, New York 10029

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- ¹ Lal, H., *Life Sci.*, **17**, 483-496 (1975).
- ² Smith, C. B., Villarreal, J. E., and Bednarczyk, J. H., *Science*, **170**, 1106-1108 (1970).
- ³ Clouet, D. H., and Ratner, M., *Science*, **168**, 854-856 (1970).
- ⁴ Kuskinsky, K., and Hornykiewicz, O., *Eur. J. Pharmacol.*, **19**, 119-122 (1972).
- ⁵ Pozuelo, J., and Kerr, F. W., *Proc. Mayo Clin.*, **47**, 621-628 (1972).
- ⁶ Gianutsos, G., Hynes, M. H., Puri, S. K., Drawbaugh, R. B., and Lal, H., *Psychopharmacologia*, **34**, 37-44 (1974).
- ⁷ Glick, S. D., Cox, R. D., and Crane, A. M., *Psychopharmacologia*, **41**, 219-224 (1975).
- ⁸ Jerussi, T. P., and Glick, S. D., *Neuropharmacology*, **13**, 283-286 (1974).
- ⁹ Glick, S. D., Jerussi, T. P., Waters, D. H., and Green, J. P., *Biochem. Pharmacol.*, **23**, 3223-3225 (1974).
- ¹⁰ Zimmerberg, B., Glick, S. D., and Jerussi, T. P., *Science*, **185**, 623-625 (1974).
- ¹¹ Jerussi, T. P., and Glick, S. D., *Psychopharmacologia*, **40**, 329-334 (1975).
- ¹² Glick, S. D., Crane, A. M., Jerussi, T. P., Fleisher, L. N., and Green, J. P., *Nature*, **254**, 616-617 (1975).
- ¹³ Terenius, L., and Wahlstrom, A., *Acta physiol. scand.*, **94**, 74-81 (1975).
- ¹⁴ Hughes, J., *Brain Res.*, **88**, 295-308 (1975).
- ¹⁵ Pasternak, G. W., Goodman, R., and Snyder, S., *Life Sci.*, **16**, 1765-1769 (1975).
- ¹⁶ Greenstein, S. D., and Glick, S. D., *Pharmac. Biochem. Behav.*, **3**, 507-510 (1975).
- ¹⁷ Glick, S. D., Zimmerberg, B., and Greenstein, S., *Brain Res.* (in the press).
- ¹⁸ Goode, P. G., *Br. J. Pharmacol.*, **41**, 558-566 (1971).
- ¹⁹ Glick, S. D., Zimmerberg, B., and Charap, A. D., *Psychopharmacologia*, **32**, 365-260 (1973).
- ²⁰ Martin, W. R., Wikler, A., Eades, C. G., and Pescor, F. T., *Psychopharmacologia*, **4**, 247-260 (1963).
- ²¹ Glick, S. D., Jerussi, T. P., and Zimmerberg, B., in *Lateralization in the Nervous System* (edit. by Harnad, S.) (Academic, New York, in the press).
- ²² Pert, C. H., and Snyder, S. H., *Science*, **179**, 1011-1014 (1973).
- ²³ Cochlin, J., and Kornetsky, J., *Pharmac. exp. Ther.*, **154**, 1-10 (1964).
- ²⁴ Zimmerberg, B., Charap, A. D., and Glick, S. D., *Nature*, **247**, 376-377 (1974).
- ²⁵ Babbini, M., Gaiardi, M., and Bartoletti, M., *Neuropharmacology*, **14**, 611-614 (1975).

Lithium differentially antagonises self-stimulation facilitated by morphine and (+)-amphetamine

IN spite of widespread use of lithium (Li) in psychiatry, the mechanism underlying its antimanic action is poorly understood. Studies of the effects of Li on spontaneous and drug-induced behavioural activity have yielded conflicting results¹⁻⁸. Electrical self-stimulation of the brain (SS) may be a promising alternative approach to such studies. This behaviour is believed to be mediated by catecholamines (reviewed in ref. 9), which have also been implicated in the action of Li¹⁰⁻¹⁴. Investigations of the effects of Li on SS have been restricted to a small range of doses and to SS in lateral hypothalamus, and the results have not been consistent^{15,16}. We have now used a wider range of doses to investigate the effects of acute and chronic Li on SS in the substantia nigra of rats.

Electrodes were implanted stereotactically in substantia nigra (2.2 mm anterior to lambda, 1.5 mm lateral to midline, 8 mm ventral to the skull surface) of 100-120-d-old male albino rats (Carworth Farms) as described before¹⁷. One week after surgery, SS tests began in operant conditioning chambers which have been described before¹⁷. The brain was stimulated by sine wave a.c. current (0.2 s duration). Rats were required to exceed a criterion performance of 1,100 bar presses per 30 min when reinforced

by optimal current intensities (typically 40-60 μ A r.m.s.). Most rats performed at considerably higher rates. All screening and drug SS sessions lasted 30 min. After completion of drug treatments, rats were killed and electrode placements were verified as before¹⁷ (Fig. 1).

Before treatment with Li, current intensity was adjusted to yield an average SS rate of between 1,200 and 1,800 bar presses per 30 min. After three consecutive test sessions in which responding remained within these limits, bacteriostatic saline or Li (1.5, 2.0 or 2.5 meq kg⁻¹) was administered as described in Fig. 2. Lithium chloride (Matheson, Coleman and Bell) was dissolved in sterile distilled water and injected subcutaneously in a volume containing 2 ml kg⁻¹. The solution was halved and injected into different sites. Skin ulceration was never observed as a result of injection of Li.

The effects of Li on rats self-stimulating at a lower rate were also investigated. After current intensity had been reduced to yield baseline SS rates between 450 and 950 bar presses per session, Li (2.0 meq kg⁻¹) was administered and testing was conducted as in Fig. 2.

Neither saline nor Li at 1.5 meq kg⁻¹ significantly changed SS from baseline responding rates (Fig. 2). The small reduction of SS induced by Li at 2.0 meq kg⁻¹, was statistically significant on days 3 and 5 of treatment, but not on days 7 and 10, suggesting the development of partial tolerance to the initial depressant effect of Li on SS. Although Li at 2.5 meq kg⁻¹ significantly reduced SS on days 3-10 of treatment, the large progressive reduction in SS was accompanied by an average weight loss of 9% in 10 d of treatment ($t=7.73$, $P<0.01$ for the comparison between body weight before Li and on day 10 of treatment). Concomitant lethargy was seen in some animals and it seemed possible that the effects of Li at 2.5 meq kg⁻¹ on SS might have been confounded with nonspecific toxic effects of this dose. In contrast, Li at 2.0 meq kg⁻¹ did not reduce body weight significantly. Gross observations failed to distinguish animals treated with Li at 2.0 meq kg⁻¹ from controls except for polyuria in some animals, as described by Schou¹⁸. The animals self-stimulating at a lower baseline rate (Fig. 2) also reduced SS on day 5 of treatment and subsequent recovery was apparent. Thus, the effects of Li on SS did not seem to be rate specific. These results are compatible with the report that Li increased electrical current thresholds for SS 24 h after injection¹⁶.

To evaluate the mechanism by which Li alters SS, its interactions with morphine and (+)-amphetamine were studied. Both morphine and (+)-amphetamine facilitate SS²⁰⁻²². Although the mechanism by which these drugs enhance SS has yet to be fully elucidated, there is some evidence that their effects on behaviour involve different neuropharmacological mechanisms^{23,24}.

Before initiation of chronic treatment with morphine, current intensity was reduced to yield SS between 450 and 950 bar presses per 30 min. After three consecutive test sessions of responding within these limits, morphine (Mallinckrodt, 15 mg kg⁻¹, subcutaneously in saline) was administered daily. After 9 d 19 of 24 rats had each increased SS to at least 50% above baseline responding. These 19 rats were then matched for response rates on day 9 of chronic treatment with morphine and were injected subcutaneously with saline, Li at 1.5 meq kg⁻¹, or Li at 2.0 meq kg⁻¹. The remaining five rats reduced SS during chronic treatment with morphine and were eliminated from the study.

Li significantly reduced morphine-facilitated SS throughout treatment (Fig. 3). At no time during combined Li (2.0 meq kg⁻¹) and morphine treatment did any animal regain the SS rate which had been attained on day 9 of treatment with morphine alone. After cessation of treatment with Li (2.0 meq kg⁻¹) on day 22, SS rates increased in five of six rats and on day 25 of continued treatment

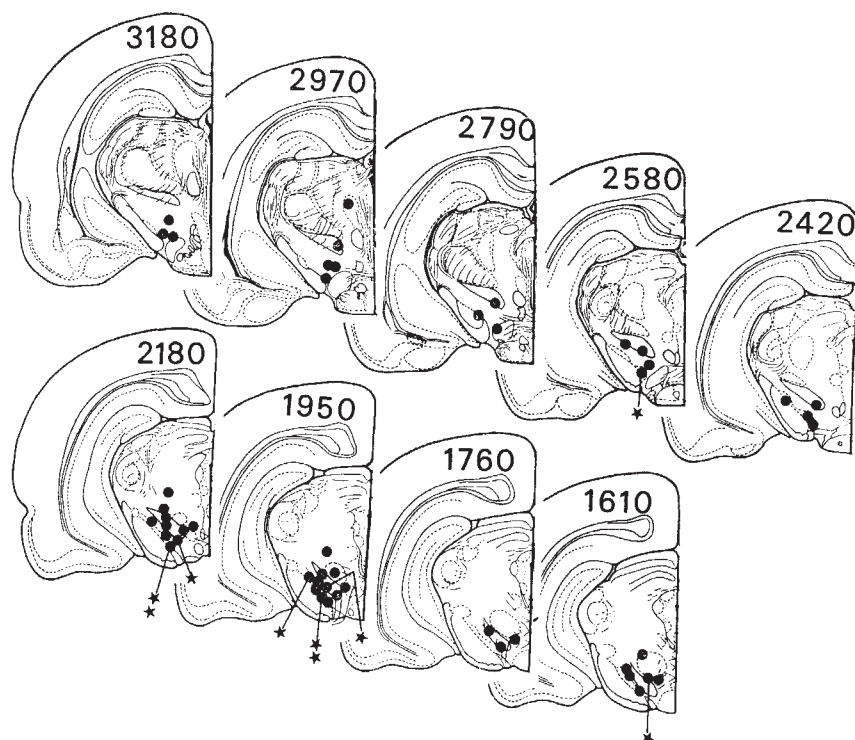
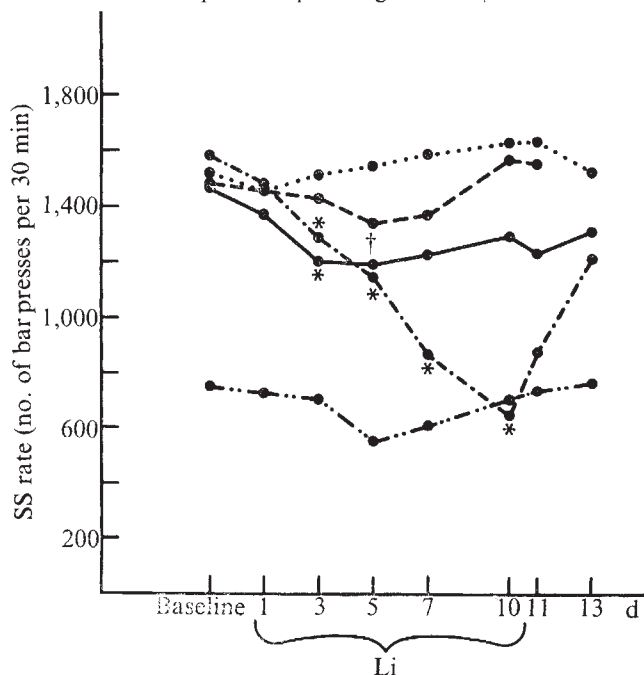


Fig. 1 Electrode placements of self-stimulating animals. Frontal sections are modified from König and Klippel¹⁸. Because of causes unrelated to the nature of the experimental treatment, histological results were unavailable for three rats. Some placements were slightly anterior to substantia nigra and others fell slightly dorsal to this structure, but there was no apparent relationship between electrode placement and experimental results. ★, Two overlapping electrode tip placements; ★★, three overlapping placements.

with morphine, SS did not differ significantly from those after morphine alone on day 9. Neither saline nor Li at 1.5 meq kg⁻¹ significantly altered morphine-facilitated SS on any day of testing.

The observed antagonism of morphine-facilitated SS by Li agrees with the report²⁵ that Li reduces oral morphine consumption in morphine-dependent rats and that tolerance does not seem to develop to this effect. One possible explanation for the effects of Li on morphine-facilitated SS is that Li interferes selectively with the penetration or

Fig. 2 Effects of chronic Li treatment on SS. Test sessions for SS took place 2 h after injection of Li on days 1, 3, 5, 7, and 10 of treatment. The rats were retested for SS on the first and third days after cessation of Li treatment (days 11 and 13). —, Saline ($n = 8$); ---, Li, 1.5 meq kg⁻¹ ($n = 8$); —, Li, 2.0 meq kg⁻¹ ($n = 8$); - · - ·, Li, 2.5 meq kg⁻¹ ($n = 6$); - · - ·, Li, 2.0 meq kg⁻¹, low rate ($n = 3$). * $P < 0.05$ for comparison between the indicated data point and preceding baseline. † $P < 0.01$.



distribution of the narcotic. This does not seem to be the case, however, because Li has been reported to enhance morphine-induced analgesia²⁶.

Before treatment with (+)-amphetamine, current intensity was reduced in the same manner as for combined treatment with morphine and Li. After responding stabilised, (+)-amphetamine (Sigma, 0.5 mg kg⁻¹, free base) was administered subcutaneously 30 min before testing (days 1–17, Fig. 4). Li was administered daily from days 5 to 14 (Fig. 4). Chronic Li (2.0 meq kg⁻¹) did not alter SS significantly on any day of treatment with Li and (+)-amphetamine (Fig. 4). Because the effects of (+)-amphetamine on SS in substantia nigra are not altered by repeated administration¹⁷, it was not necessary to control for this possibility.

The lack of antagonism of (+)-amphetamine-facilitated SS by Li contrasts with the reported reduction of (+)-amphetamine-induced hyperactivity by Li⁸. These results further support the suggestion that different neurochemical mechanisms mediate these behavioural activities²².

Because both the nigro-neostriatal dopamine pathway^{22,27,28} and the dorsal and ventral noradrenaline pathways^{29,30} have been proposed as substrates for SS in substantia nigra, it is not possible to state which of these pathways are involved in the effects reported here. Regardless of the pathway involved, these results suggest that (+)-amphetamine- and morphine-induced facilitation of SS are mediated by different mechanisms of catecholamine neurotransmission. Enhancement of activity and SS by low doses of (+)-amphetamine is believed to result from facilitation of catecholamine release^{20,24,31} from a newly synthesised pool^{32,35}. In contrast, the effects of morphine on activity seem to depend on a reserpine-sensitive pool of stored catecholamines^{24,36}. If, therefore, Li interferes with catecholamine storage as suggested¹², morphine- but not (+)-amphetamine-facilitated SS would be expected to be reduced by Li.

Regardless of the neurochemical mechanisms involved, the differential effects of Li on morphine- and (+)-amphetamine-induced facilitation of SS may have important clinical implications. If the drug abuse potential of morphine and amphetamine is related to the activation of brain reward pathways, then the results obtained with

Fig. 3 Effects of chronic Li treatment on morphine-facilitated SS. Morphine was injected daily (days 1–25). Test sessions for SS took place on alternate days 3 h after injection of morphine. From days 11 to 21 Li was administered daily 1 h after morphine. The effects of Li on SS facilitated by morphine were assessed by comparison with responding on day 9., Saline+morphine ($n = 7$); ---, Li, 1.5 meq kg^{-1} +morphine ($n = 6$); —, Li, 2.0 meq kg^{-1} +morphine ($n = 6$). * $P < 0.05$ for the difference between the data point and the corresponding score on day 9. † $P < 0.01$.

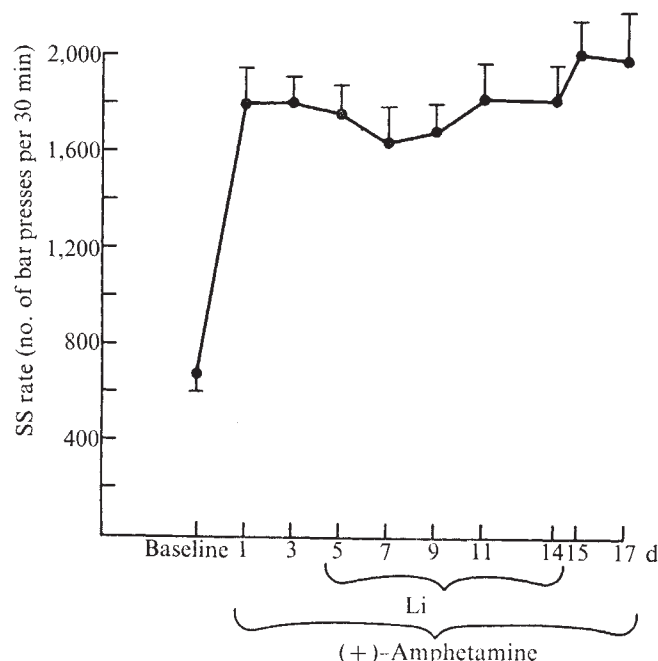
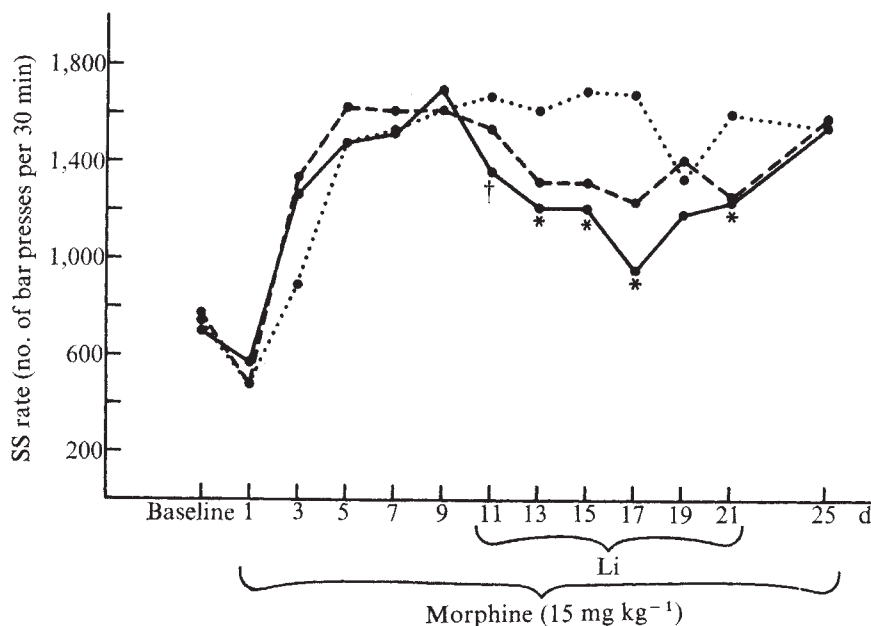


Fig. 4 Effects of chronic Li treatment on (+)-amphetamine-facilitated SS ($n = 8$). (+)-Amphetamine was administered 30 min before each test session on days 1–17. Li (2.0 meq kg^{-1}) was administered daily 2 h before testing on days 5–14. Self-stimulation during combined Li and (+)-amphetamine administration was compared with responding on day 3.

SS may have practical applications. Thus, for example, on the basis of our results it may be suggested that Li will be effective in the treatment of morphine, but not amphetamine, addiction.

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JEFFREY M. LIEBMAN
DAVID S. SEGAL

Department of Psychiatry,
School of Medicine,
University of California, San Diego,
La Jolla, California 92093

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† Matussek, N., and Linsmayer, M., *Life Sci.*, 7, 371–375 (1968).

- 2 D'Encarnacao, P. S., and Anderson, K., *Dis. Nerv. Syst.*, 31, 494–496 (1970).
- 3 Cox, C., Harrison-Read, P. E., Steinberg, H., and Tomkiewicz, M., *Nature*, 232, 336–338 (1971).
- 4 Johnson, F. N., and Wormington, S., *Nature*, 235, 159–160 (1972).
- 5 U'Prichard, D. C., and Stein, H., *Br. J. Pharmac.*, 44, 349–350P (1972).
- 6 Smith, D. F., and Smith, H. B., *Psychopharmacologia*, 30, 83–88 (1973).
- 7 Poitou, P., Boulu, R., and Bohoun, C., *Experientia*, 31, 99–101 (1975).
- 8 Segal, D. S., Callaghan, M., and Mandell, A. J., *Nature*, 254, 58–59 (1975).
- 9 German, D. C., and Bowden, D. M., *Brain Res.*, 73, 381–419 (1974).
- 10 Corrodi, H., Fuxe, K., and Schou, M., *Life Sci.*, 8, 643–651 (1969).
- 11 Katz, R. I., and Kopin, I. J., *Biochem. Pharmac.*, 18, 1935–1939 (1969).
- 12 Greenspan, K., Aronoff, M. S., and Bogdanski, D. F., *Pharmacology*, 3, 129–136 (1970).
- 13 Friedman, E., and Gershon, S., *Nature*, 243, 520–521 (1973).
- 14 Schildkraut, J. J., in *Lithium: Its Role in Psychiatric Research and Treatment* (edit. by Gershon, S., and Shopsin, B.), 51–73 (Plenum, New York, 1973).
- 15 Ramsey, T. A., Mendels, J., Hamilton, C., and Frazer, A., *Life Sci.*, 11, 773–779 (1972).
- 16 Pick-Cassens, G., and Mills, A. W., *Psychopharmacologia*, 30, 283–290 (1973).
- 17 Liebman, J. M., and Segal, D. S., *Behav. Biol.* (in the press).
- 18 König, J. F. R., and Klippel, R. A., in *The Rat Brain* (Krieger, Huntington, New York, 1967).
- 19 Schou, M., *Acta pharmac. tox.*, 15, 70–84 (1958).
- 20 Stein, L., *Fedn Proc.*, 23, 836–850 (1964).
- 21 Lorens, S. A., and Mitchell, C. L., *Psychopharmacologia*, 32, 271–277 (1973).
- 22 Liebman, J. M., and Butcher, L. L., *Naunyn-Schmied. Arch. Pharmac.*, 284, 167–194 (1974).
- 23 Ayhan, I. H., and Randrup, A., *Psychopharmacologia*, 29, 317–328 (1973).
- 24 Villarreal, J. E., Guzman, M., and Smith, C. B., *J. Pharmac. exp. Ther.*, 187, 1–7 (1973).
- 25 Tomkiewicz, M., and Steinberg, H., *Nature*, 252, 227–229 (1974).
- 26 Jensen, J., *Acta pharmac. tox.*, 35, 395–402 (1974).
- 27 Crow, T. J., *Brain Res.*, 36, 265–273 (1972).
- 28 Phillips, A. G., and Fibiger, H. C., *Science*, 179, 575–577 (1973).
- 29 Belluzzi, J. D., Ritter, S., Wise, C. D., and Stein, L., *Behav. Biol.*, 13, 103–111 (1975).
- 30 Ritter, S., and Stein, L., *Brain Res.*, 81, 145–157 (1974).
- 31 Stolck, J. M., and Rech, R. H., *J. Pharmac. exp. Ther.*, 158, 140–149 (1967).
- 32 Carlsson, A., in *Amphetamine and Related Compounds* (edit. by Costa, E., and Garattini, S.), 289–300 (Raven, New York, 1970).
- 33 Fuxe, K., and Ungerstedt, U., in *Amphetamine and Related Compounds* (edit. by Costa, E., and Garattini, S.), 257–288 (Raven, New York, 1970).
- 34 Franklin, K. B. J., and Herberg, L. J., *Brain Res.*, 67, 429–437 (1974).
- 35 Chiuheh, C. C., and Moore, K. E., *J. Pharmac. exp. Ther.*, 192, 642–653 (1975).
- 36 Carroll, B. J., and Sharp, P. T., *Science*, 172, 1355–1357 (1971).

Histamine-sensitive adenylate cyclase in mammalian brain

THERE is increasing interest in the possibility that histamine may be a neurotransmitter in the mammalian central nervous system (CNS). Electrophysiological evidence supports the existence of histamine receptors in nervous tissue through which neuronal excitability can be regulated. For example, the firing rate of certain neurones in the brain is depressed by histamine and this depression is specifically blocked by the H_2 antagonist, metiamide¹. In addition, histamine influences the electrical excitability of neurones in the superior cervical ganglion². Certain neurotransmitters may exert some of their effects in nervous tissue by stimulating the formation of cyclic AMP. It is therefore of interest that histamine raises cyclic AMP levels in brain slices^{3–7}, as do several other putative neurotransmitters, such as noradrenaline, dopamine and 5-hydroxytryptamine (5-HT). Furthermore, a dopamine-sensitive adenylate cyclase

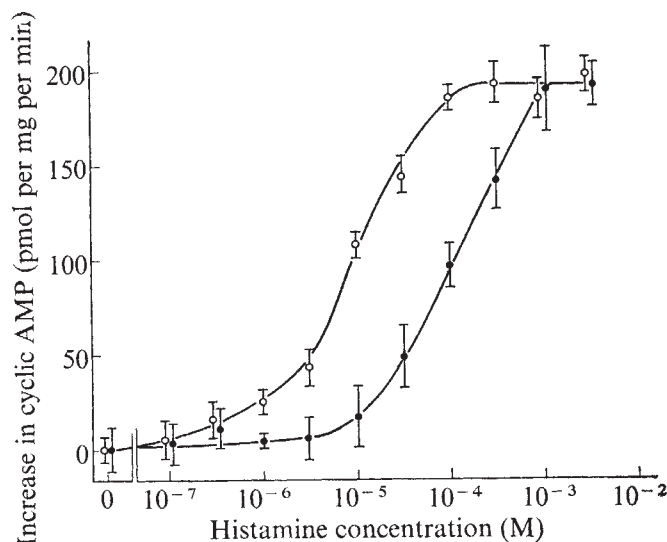


Fig. 1 Effect of various concentrations of histamine in the absence (○) or presence (●) of 10^{-5} M metiamide, on adenylate cyclase activity in homogenates of dorsal hippocampus from guinea pig brain. Adult female Hartley guinea pigs were killed by decapitation. The dorsal hippocampi were dissected, weighed, and manually homogenised in 150 volumes ice-cold 2 mM Tris maleate-2 mM EGTA, pH 7.8, using a glass homogeniser with a Teflon pestle. The adenylate cyclase reaction mixture contained the following final concentrations (mM): Tris maleate (pH 7.8), 100; EGTA, 0.6; 3-isobutyl-1-methylxanthine, 1; $MgCl_2$, 2; ATP, 1; GTP, 0.1; plus test substances as indicated, in a final volume of 500 μ l. The enzyme reaction, initiated by the addition of 50 μ l homogenate, was carried out for 6 min at 30 °C, and was terminated by placing the assay tubes in a boiling water bath for 2 min. Aliquots (50 μ l) were assayed in duplicate for cyclic AMP by the method of Brown *et al.*¹². Protein was determined by the method of Lowry *et al.*¹³. In the absence of added histamine or metiamide, 153 ± 4 pmol cyclic AMP per min per mg protein were formed; in the presence of 10^{-5} M metiamide, 152 ± 10 pmol cyclic AMP per min per mg protein were formed. The increase in cyclic AMP above basal level is plotted as a function of histamine concentration. Values represent the mean $\pm$ s.e.m. of nine replicate samples in the absence, and three replicate samples in the presence, of histamine.

has been found in homogenates of rat caudate nucleus and olfactory tubercle, with properties very similar to those of the dopamine receptor in mammalian brain⁸⁻¹⁰. We report here the occurrence of an adenylate cyclase which is activated by low concentrations of histamine in several regions of guinea pig brain. This histamine-sensitive adenylate cyclase has the pharmacological properties of an H_2 receptor. The results indicate that the actions of histamine on H_2 receptors in the CNS may be mediated through cyclic AMP.

In the present study, 100 μ M histamine caused maximal stimulation of adenylate cyclase in homogenates of guinea pig dorsal hippocampus. In several experiments, the maximal increase in adenylate cyclase activity varied from 70–200%. Addition of GTP to the reaction mixture increased enzyme activity and markedly potentiated the stimulation of adenylate cyclase by histamine. There results are consistent with other studies indicating that GTP and its analogues can regulate the activity of adenylate cyclases¹¹.

Figure 1 illustrates the effects of various concentrations of histamine on adenylate cyclase activity in homogenates of dorsal hippocampus from guinea pig brain. The concentration of histamine required for half-maximal activation (K_a) of the enzyme was 8 μ M. This agrees well with the K_a values of 7 and 9 μ M reported, respectively, for histamine-induced cyclic AMP formation in brain slices⁶ and in cultures of intact human astrocytoma cells¹⁴.

Two types of histamine receptors have been characterised in mammalian tissues^{15,16}. H_1 receptors are selectively blocked by classical anti-histaminic agents such as mepyramine and promethazine, whereas H_2 receptors are selectively blocked by recently developed H_2 antagonists such as metiamide, cime-

tidine and burimamide¹⁷. As shown in Fig. 1, metiamide acted as a competitive inhibitor of the activation by histamine of adenylate cyclase. In the presence of 10 μ M metiamide, the apparent K_a for histamine was increased to 100 μ M. From these data, the K_i value¹⁸ of the enzyme for metiamide was calculated to be 0.87 μ M, which is in excellent agreement with the K_i values for metiamide inhibition of H_2 receptors calculated from measurements of physiological responses using guinea pig atrium (0.92 μ M) and rat uterus (0.75 μ M)¹⁷. In contrast, the H_1 antagonist mepyramine, at 1 μ M (a concentration which maximally inhibits the effects of 100 μ M histamine on H_1 receptors⁶), had no effect either on the K_a for histamine or on the maximal stimulation of adenylate cyclase. Other agents which antagonise the actions of various neurotransmitters on their receptors were tested for possible effects on adenylate cyclase activity in the absence and presence of 100 μ M histamine—propranolol, phentolamine, atropine, and fluphenazine, in concentrations of 10 μ M, had no effect on basal or histamine-stimulated enzyme activity.

The ability of two substances known to act as agonists at histamine receptors were compared with histamine as activators of adenylate cyclase (Fig. 2). 4-Methyl-histamine increased adenylate cyclase activity with half-maximal stimulation occurring at 23 μ M. This compound has been reported to have 25–43% of the potency of histamine in activating H_2 receptors, but negligible activity at H_1 receptors¹⁶. In contrast, 2-methyl-histamine primarily affects H_1 receptors. It weakly activates H_2 receptors as well, having 2–4% of the potency of histamine in this regard. In agreement with these properties, 2-methyl-histamine stimulated adenylate cyclase, but only at high concentrations ($K_a = 120$ μ M). Thus, the pharmacological properties of the histamine-sensitive adenylate cyclase seem to be those of an H_2 receptor.

The regional distribution of histamine-sensitive adenylate cyclase activity in rat and guinea pig brain is shown in Table 1. In guinea pig brain, histamine markedly stimulated adenylate cyclase in homogenates from hippocampus, neocortex, and corpus striatum, but did not significantly affect enzyme activity in other brain regions. This localisation of histamine-sensitive adenylate cyclase activity correlates well with the regional distribution of histamine-induced cyclic AMP accumulation in brain slices⁷. Of the various regions of rat brain examined, histamine-stimulated adenylate cyclase only in neocortex, where a small but statistically significant increase in activity was seen. An histaminergic pathway which projects to the cortex in rat brain has recently been described¹⁹. The histamine-sensitive adenylate cyclase present in rat neocortex may represent the postsynaptic receptor of neurones in this region receiving

Fig. 2 Effect of various concentrations of histamine (○), 4-methyl-histamine (△), and 2-methyl-histamine (×) on adenylate cyclase activity in homogenates of guinea pig dorsal hippocampus. Experimental procedures were as in Fig. 1. The maximal stimulation of adenylate cyclase activity by saturating concentrations of histamine, 4-methyl-histamine, and 2-methyl-histamine was the same within the limits of experimental error.

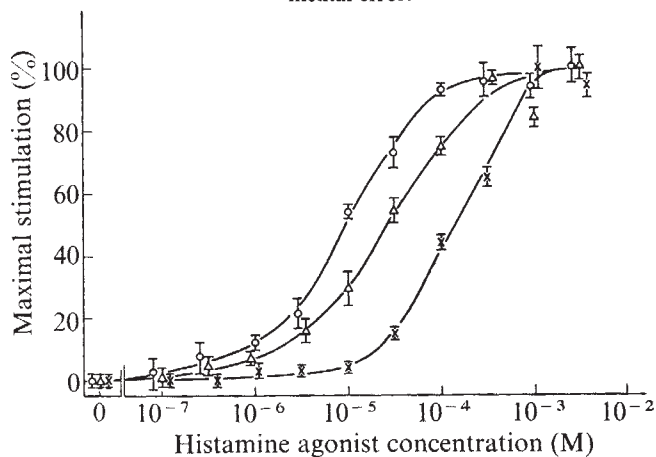


Table 1 Regional distribution of histamine-sensitive adenylate cyclase activity in guinea pig and rat brain

Brain region	Adenylate cyclase activity* (pmol per min per mg protein)	
	-Histamine	+Histamine (100 μ M)
Guinea pig		
Dorsal hippocampus	106 $\pm$ 4	218 $\pm$ 8†
Ventral hippocampus	111 $\pm$ 6	199 $\pm$ 6†
Neocortex	98 $\pm$ 4	187 $\pm$ 7†
Corpus striatum	101 $\pm$ 5	165 $\pm$ 3†
Cerebellum	33 $\pm$ 3	35 $\pm$ 2
Hypothalamus	157 $\pm$ 4	158 $\pm$ 6
Thalamus	65 $\pm$ 5	71 $\pm$ 3
Midbrain	127 $\pm$ 4	129 $\pm$ 3
Pons	159 $\pm$ 8	156 $\pm$ 5
Medulla	118 $\pm$ 6	125 $\pm$ 4
Optic chiasm	46 $\pm$ 9	44 $\pm$ 6
Rat		
Hippocampus	63 $\pm$ 3	67 $\pm$ 6
Neocortex	54 $\pm$ 2	65 $\pm$ 3†
Corpus striatum	72 $\pm$ 6	74 $\pm$ 3
Cerebellum	73 $\pm$ 5	78 $\pm$ 6

*Experimental procedures were as described in Fig. 1. Values represent mean $\pm$ s.e.m. of four replicate samples.

†Activity in the presence of histamine was significantly different ($P < 0.005$) from that in the absence of histamine.

histaminergic innervation. The localisation of histaminergic pathways in guinea pig brain has not yet been determined.

The non-uniform distribution of histamine-sensitive adenylate cyclase activity in brain is of particular interest, since histamine has been shown to increase cyclic AMP in cultured cells derived from glial elements¹⁴ as well as to increase adenylate cyclase activity in brain capillaries²⁰. If the enzyme we are studying were primarily glial or vascular in origin, one would expect a fairly uniform distribution of histamine-sensitive adenylate cyclase activity throughout the brain. The non-uniform distribution of activity actually observed (Table 1) is more consistent with the possibility that the enzyme is located primarily in selected neurones which receive histaminergic innervation.

In studies of guinea pig neocortical and hippocampal brain slices, activation of either H_1 or H_2 receptors led to the accumulation of cyclic AMP^{6,7}. The present results indicate that the H_2 receptor in brain, like the dopamine receptor⁸⁻¹⁰, is intimately associated with an adenylate cyclase. The idea of a direct connection between H_2 receptors and adenylate cyclase in nervous tissue is supported by studies of heart^{21,22} and stomach^{23,24}, in which histamine, acting on H_2 receptors, caused an increase in formation of cyclic AMP in both intact preparations and homogenates.

We have been unable, using homogenates of guinea pig hippocampus, to identify a histamine-sensitive adenylate cyclase which possesses the pharmacological properties of an H_1 receptor. It is possible that such an adenylate cyclase does exist in brain, but that it was either labile or inactive in the experimental conditions used. Alternatively, histamine, acting on H_1 receptors, may cause cyclic AMP accumulation in brain slices by an indirect mechanism. For example, histamine might cause the release of other neurotransmitters which, acting on their own target cells, could then activate adenylate cyclases. Clearly, the relationship of H_1 receptors to cyclic AMP metabolism requires further investigation. Interestingly, in non-neuronal tissues, an increase in cyclic GMP in response to activation of H_1 receptors has been reported^{25,26}.

The present results indicate that the H_2 receptor in mammalian brain may be the histamine-binding portion of a histamine-sensitive adenylate cyclase. One corollary of this conclusion is that the physiological consequences of histamine acting at H_2 receptors in the brain, such as depression of the firing rate of certain neurones¹, may be mediated through cyclic AMP. Further studies on the biochemistry and pharmacology of this histamine-sensitive adenylate cyclase, and its

relationship to physiological processes in neurones, may help to clarify the role of histamine in the mammalian nervous system.

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LINDA R. HEGSTRAND
PHILIP D. KANOF
PAUL GREENGARD

Department of Pharmacology,
Yale University School of Medicine,
333 Cedar Street,
New Haven, Connecticut 06510

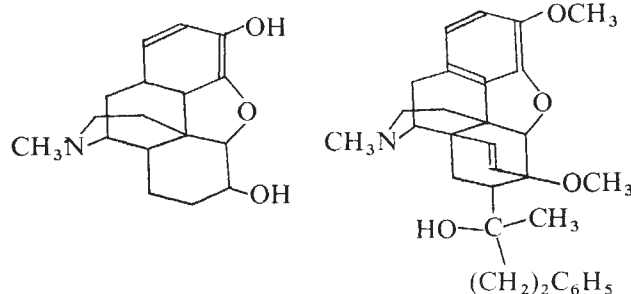
Received December 9, 1975; accepted January 6, 1976.

- Haas, H. L., and Bucher, U. M., *Nature*, **225**, 634-635 (1975).
- Brimble, M. J., and Wallis, D. I., *Nature*, **246**, 156-158 (1973).
- Kakiuchi, S., and Rall, T. W., *Molec. Pharmacol.*, **4**, 367-388 (1968).
- Schultz, J., and Daly, J. W., *J. Neurochem.*, **21**, 573-579 (1973).
- Chasin, J., Mamrak, F., and Samaniego, S. G., *J. Neurochem.*, **22**, 1031-1038 (1974).
- Baudry, M., Martres, M.-P., and Schwartz, J.-C., *Nature*, **253**, 362-363 (1975).
- Rogers, M., Dismukes, K., and Daly, J. W., *J. Neurochem.*, **25**, 531-534 (1975).
- Kebabian, J. W., Petzold, G. L., and Greengard, P., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2145-2149 (1972).
- Clement-Cormier, Y. C., Kebabian, J. W., Petzold, G. L., and Greengard, P., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1113-1117 (1974).
- Iversen, L. L., *Science*, **188**, 1084-1089 (1975).
- Rodbell, M., *J. biol. Chem.*, **250**, 5826-5834 (1975).
- Brown, B. L., Albano, J. D. M., Ekins, R. P., and Sghersi, A. M., *Biochem. J.*, **121**, 561-562 (1971).
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265-275 (1951).
- Clark, R. B., and Perkins, J. P., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2757-2760 (1971).
- Ash, A. S. F., and Schild, H. O., *Br. J. Pharmac. exp. Chemother.*, **27**, 427-439 (1966).
- Black, J. W., Duncan, W. A. M., Durant, G. J., Ganellin, C. R., and Parsons, E. M., *Nature*, **236**, 385-390 (1972).
- Black, J. W., Durant, G. J., Emmett, J. C., and Ganellin, C. R., *Nature*, **248**, 65-67 (1974).
- Chen, Y.-C., and Prusoff, W. H., *Biochem. Pharmacol.*, **22**, 3099-3108 (1973).
- Garbarg, M., Barbin, G., Feger, J., and Schwartz, J.-C., *Science*, **186**, 833-835 (1974).
- Joó, F., Rakonczay, Z., and Wollemann, M., *Experientia*, **31**, 582-584 (1975).
- Klein, I., and Levey, G. S., *J. clin. Invest.*, **51**, 1012-1015 (1971).
- McNeill, J. H., and Verma, S. C., *J. Pharmac. exp. Ther.*, **188**, 180-188 (1974).
- Domschke, W., Domschke, S., Classen, M., and Demling, L., *Nature*, **241**, 454-455 (1973).
- Dousa, T. P., and Code, C. F., *J. clin. Invest.*, **53**, 334-337 (1974).
- Schultz, G., Hardman, J. G., Schultz, K., Baird, C. E., and Sutherland, E. W., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3889-3893 (1973).
- Clyman, R. L., Sandler, J. A., Manganiello, V. C., and Vaughan, M., *J. clin. Invest.*, **55**, 1020-1025 (1975).

Biosynthetic origin and receptor conformation of methionine enkephalin

RECENT reports have shown that the brain contains an endogenous peptide with opiate-like activity¹⁻³ and similar peptides have been found in the pituitary^{4,5,13}. One of the brain peptides, known as methionine enkephalin, was identified as a pentapeptide Tyr-Gly-Gly-Phe-Met⁶, and evidence was presented that a minor component may have leucine in place of methionine. The principal sequence is identical to that at the NH_2 -terminus of lipotropin C fragment, a peptide discovered in substantial quantity in porcine pituitary^{7,8}. This suggests that methionine enkephalin is derived *in vivo* by proteolytic cleavage of C fragment. Since enkephalin is thought to compete directly with opiates for the brain opiate receptor, while having no primary structure similarity to opiates, we have searched for and found a basis for the competition in a proposed secondary structure for the peptide.

Spatial models of morphine (I), oripavine (II), a potent derivative of morphine, and methionine enkephalin were



constructed. The conformation of morphine is essentially defined by the rigidity of its molecular structure and similar considerations apply to the assignment of conformation in oripavine. The pentapeptide, on the other hand, should be able to assume various conformations in aqueous solution but at the receptor a unique conformation would be expected. With methionine enkephalin a model could be constructed which presents structural elements in common with morphine; the proposed structure bears a striking resemblance to the spatial structure of oripavine (Fig. 1).

In the models, specific functional groups are held in the same orientation whether suspended from a peptide backbone or forming part of a rigid alkaloid structure. The tyrosine side chain and the amino group in enkephalin correspond exactly with the orientations of the phenolic ring in the phenanthrene nucleus and the tertiary amino group of oripavine. The two glycine residues occupy positions 2 and 3 in a β bend with a hydrogen bond between the carbonyl group of the tyrosine residue and the amino group of the phenylalanine, allowing the phenylalanine side chain to coincide with the positioning of the phenylethyl substituent at position 19 in the alkaloid. This lipophilic group, which is absent in morphine, could contribute to the stronger receptor binding of oripavine and enkephalin. The carboxyl terminal methionine residue lies between the aromatic side chains of tyrosine and phenylalanine; the methyl thioether chain then has the same orientation as the methyl ether substituent at position 6 in oripavine.

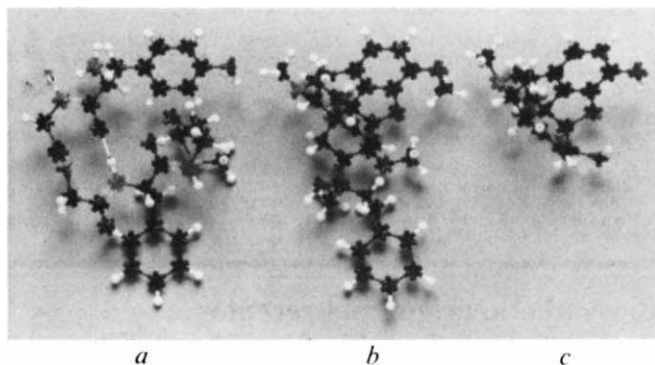


Fig. 1 Framework molecular models of tyrosylglycylglycyl-phenylalanylmethionine (methionine enkephalin) (a); oripavine (b), and morphine (c). The models were constructed from components described by Doré¹².

Evidence for the proposed conformation of the neuroactive pentapeptide emerged on application of empirical rules for predicting secondary structure from a knowledge of amino acid sequence. Both the Chou and Fasman rules^{9,10} and the principles of Lewis, Momany and Scheraga¹¹ indicated a strong tendency for the NH_2 -terminal region of the C fragment of lipotropin to form a β bend comprising the residues Tyr-Gly-Gly-Phe and this β bend is one of the structural elements in a model of lipotropin constructed in our laboratory (unpublished results). It is notable that the conformation proposed for the biologically active form of methionine enkephalin centres on a β bend involving the same four residues.

The molecular structures proposed for the opiate reactive peptide, morphine and oripavine provide an explanation for the fact that a characteristic biological activity is expressed by molecules entirely different in class and origin.

A. F. BRADBURY
D. G. SMYTH
C. R. SNELL

National Institute for Medical Research,
Mill Hill, London NW7 1AA, UK

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¹ Terenius, L., and Wahlström, A., *Life Sci.*, **16**, 1759–1764 (1975).

- ² Hughes, J., *Brain Res.*, **88**, 295 (1975).
- ³ Pasternak, G. W., Goodman, R., and Snyder, S. H., *Life Sci.*, **16**, 1765–1769 (1975).
- ⁴ Teschemacher, H., Opheim, K. E., Cox, B. M., and Goldstein, A., *Life Sci.*, **16**, 1771–1776 (1975).
- ⁵ Cox, B. M., Opheim, K. E., Teschemacher, H., and Goldstein, A., *Life Sci.*, **16**, 1777–1782 (1975).
- ⁶ Hughes, J., et al., *Nature*, **258**, 577–579 (1975).
- ⁷ Bradbury, A. F., Smyth, D. G., and Snell, C. R., in *Peptides: Chemistry, Structure and Biology* (edit. by Meienhofer, J.) (Ann Arbor Science Inc., in the press).
- ⁸ Bradbury, A. F., Smyth, D. G., and Snell, C. R., in *The Polypeptide Hormones: Molecular and Cellular Aspects*, Ciba Foundation Symp. No. 41 (edit. by Fitzsimons, D. W.), 61–69 (Associated Scientific, 1976).
- ⁹ Chou, P. Y., and Fasman, G. D., *Biochemistry*, **13**, 211–222 (1974).
- ¹⁰ Chou, P. Y., and Fasman, G. D., *Biochemistry*, **13**, 222–245 (1974).
- ¹¹ Lewis, P. N., Momany, F. A., and Scheraga, H. A., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2293–2297 (1971).
- ¹² Doré, C. F., *Educ. Chem.*, **7**, 194–195 (1970).
- ¹³ Bradbury, A. F., Smyth, D. G., Snell, C. R., Birdsall, N. J. M., and Hulme, E. C., *Nature* (in the press).

Gibberellic acid enhances the level of translatable mRNA for α -amylase in barley aleurone layers

α -AMYLASE is synthesised *de novo* in response to the plant hormone gibberellic acid (GA_3) in the aleurone layers of barley grains¹. This enzyme induction depends on RNA and protein synthesis, as determined by the use of inhibitors^{2–4}, and it occurs after a temperature-dependent lag period⁵. The biochemical events of the lag period have been studied with respect to ionic requirements^{6–7}, early protein synthesis³, RNA synthesis^{8–12}, polysome formation¹³ and lipid synthesis and turnover^{14–17}. It has been postulated that new mRNA for α -amylase synthesis might be produced in response to the hormone^{2,3} and there is evidence that GA_3 enhances the incorporation of labelled ribonucleosides into poly(A)-containing RNA of barley aleurone layers^{18–20}. Stimulation could be detected about 4 h after hormone treatment and although GA_3 probably stimulates synthesis of a limited number of proteins, there was stimulation of a wide size range of poly(A)-containing RNAs. Thus it was not possible to say that GA_3 had a selective effect on the synthesis of the mRNA for α -amylase.

In an attempt to relate GA_3 -stimulated α -amylase production to the level of α -amylase mRNA, we have assayed for this mRNA by translating total and poly(A)-containing RNA isolated from aleurone layers in a cell-free system derived from wheat embryos²¹. This was followed by immunoprecipitation²² and sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis²³ of the cell-free translation products. We found that the level of translatable mRNA for α -amylase increases in hormone-treated tissue in parallel with the increased rate of enzyme synthesis.

The template activity of total RNA from both control and hormone-treated tissue was low (fivefold stimulation) compared with tobacco mosaic virus (TMV) RNA²⁴ (50-fold stimulation). The aleurone RNA preparations, however, did not contain inhibitors of translation when assayed with TMV RNA-directed cell-free protein synthesis (unpublished). Total RNA isolated from aleurone layers incubated in the absence and presence of GA_3 for 8 h was used to program the wheat embryo cell-free system. The translation products were analysed by SDS-polyacrylamide gel electrophoresis followed by fluorography²⁵ of the dried gels and they varied in size from 8,000 to 70,000 daltons (Fig. 1a and b). In spite of the variety of proteins induced in aleurone cells by GA_3 , there were relatively few detectable differences in the patterns of cell-free translation products when the system was programmed by RNA from GA_3 -treated and untreated tissue. But cell-free synthesis of at least two polypeptides was increased by RNA from GA_3 -treated tissue, one migrating as a broad band at about 35,000 daltons and one at 45,000 daltons. The former was not identified and may consist of several polypeptides. The 45,000-dalton polypeptide coelectrophoresed with α -amylase.

Poly(A)-RNA was fractionated from total RNA of 8-h treated aleurone layers by oligo(dT)-cellulose chromato-

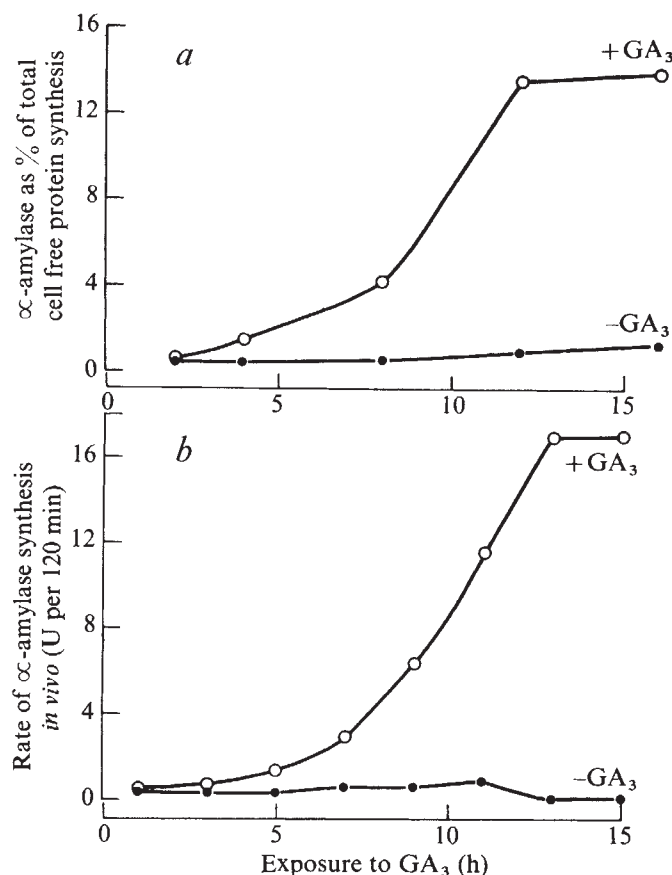


Fig. 1 Fluorographs of SDS-polyacrylamide gel electrophoresis of cell-free translation products of RNA isolated from aleurone layers 8 h after the beginning of hormone treatment. Aleurone layers were prepared from barley (var. Himalaya) half-seeds and incubated as before⁴, at 25 °C in medium containing 10⁻² M CaCl₂ and 10⁻⁶ M GA₃ where required. Total RNA was prepared by the following modification of the method of Click and Hackett²⁵. The layers were ground in 10 ml of buffer containing

0.1 M sodium glycinate, pH 9.5, 0.01 M EDTA, 0.1 M sodium chloride and 1% SDS, with 1% insoluble polyvinylpyrrolidone (L. Taiz, personal communication). After centrifugation at 10,000g for 10 min an equal volume of phenol-chloroform (1:1) was added to the supernatant and the aqueous phase was extracted for 5 min while being shaken. The phases were separated, the phenol phase was shaken for a further 5 min with 5 ml of the same buffer and the combined aqueous phases were then extracted with 5 ml of phenol-chloroform. The RNA in the aqueous phase was precipitated by addition of 2.5 volumes of ethanol and stored overnight at -20 °C. It was then pelleted by centrifugation, redissolved in 3 ml of 0.5 M sodium acetate, pH 5.8 and reprecipitated with 0.4 ml of 1% acetyltrimethylammonium bromide²⁷. The precipitate was recovered by centrifugation and washed with 0.1 M sodium acetate in 70% ethanol and finally with 3 M sodium acetate, 5 mM EDTA, pH 5.0. The RNA was then used directly or fractionated on oligo(dT)-cellulose as before²⁸ except that SDS (0.1%) was included in the buffer. After precipitation, the RNA was used to program the wheat embryo cell-free protein synthesising system²¹ as modified²⁴. ³⁵S-methionine (50 Ci mol⁻¹, Amersham) was the source of radioactive amino acid. The reaction was stopped after 90 min at 25 °C. The ribosomes were removed by centrifugation at 30,000 r.p.m. for 60 min and the soluble proteins were either analysed on SDS-polyacrylamide gels²³ (14%) in the presence of 2-mercaptoethanol with the modifications specified²⁴ or subjected to immunoprecipitation followed by SDS-polyacrylamide gel electrophoresis of the antibody precipitates. Sliced and stained gels were prepared for fluorography according to Bonner and Laskey²⁶. The dried gels were exposed to Kodak X-OMAT X-ray film for 7 d at -80 °C. Immunoprecipitation was carried out on the proteins of the cell-free system from which the ribosomes had been removed. Antiserum (10 µl) produced in rabbits against purified α-amylase^{31,32} was added to each reaction mixture and incubated at 25 °C for 60 min. Sufficient goat anti-rabbit gammaglobulin serum (provided by Dr David Kemp) was then added to precipitate all the rabbit antibodies and the mixture was incubated for 60 min at 25 °C and then overnight at 4 °C. The precipitates were collected by centrifugation through sucrose cushions and washed twice with saline-detergent as described³³ and modified²⁴. The immunoprecipitates were then analysed on SDS-polyacrylamide gels and prepared as described above. Cell-free translation products of total RNA: a, -GA₃; b, +GA₃. Products of poly(A)-RNA: c, -GA₃; d, +GA₃. Specific anti-α-amylase immunoprecipitates of translation products of total RNA: e, -GA₃; f, +GA₃. The numbers refer to the molecular weights (× 10⁻³) of the markers, bovine serum albumin (molecular weight 68,000), ovalbumin (molecular weight 45,000), chymotrypsinogen (molecular weight 25,000) and cytochrome c (molecular weight 12,400). The arrow denotes the position to which authentic α-amylase migrated, and 0 refers to the origin.

graphy²⁸ and was used to program the cell-free system. This step resulted in a tenfold increase in template specific activity. The patterns of cell-free translation products were identical with those obtained with total RNA (Fig. 1c and d compared with Fig. 1a and b). Our observations thus provide further evidence^{29,30}, that the poly(A)-RNA of plants includes RNA with messenger activity.

Evidence for the identification of the 45,000-dalton polypeptide synthesised *in vitro* was provided by immunoprecipitation with monospecific antibodies produced in rabbits against barley α-amylase^{31,32}. The double antibody technique was used to precipitate the anti-α-amylase²². Although the unfractionated translation products of total RNA were complex (Fig. 1a and b) the immunoprecipitation step yielded a single polypeptide which coelectrophoresed with or was slightly larger than authentic α-amylase run on the same gel and stained with Coomassie blue (Fig. 1f). Sufficient material was not available for tryptic fingerprint analysis and it was therefore not certain whether the cell-free product was identical with the authentic protein or whether it contained some extra amino acids at either the NH₂- or -COOH terminals. A study of the cell-free synthesis of immunoglobulin light chain showed that the *in vitro* product was larger than the polypeptide found *in vivo*³⁴. RNA isolated from aleurone layers incubated without hormone did not direct the cell-free synthesis of detectable α-amylase as determined by immunoprecipitation (Fig. 1e).

Based on the above evidence, we tentatively concluded

that GA₃ increased the level of mRNA for α-amylase in barley aleurone layers. We then investigated the time course of appearance of the RNA in the tissue by assaying with the cell-free translation system. Total RNA was isolated from aleurone layers incubated with and without hormone for 2, 4, 8, 12 and 16 h and was used to direct cell-free protein synthesis. There was two- to threefold variation in template efficiency of RNA from batch to batch. There was, however, no consistent difference between RNAs isolated from hormone-treated and untreated tissue or between RNAs extracted from tissue after exposure to the hormone for different times. Fluorographs of the cell-free translation products programmed by RNA extracted from tissue incubated with and without GA₃ for 2–16 h are shown in Fig. 2. The range of polypeptides was similar to those shown in Fig. 1a and b and the major difference between the RNA-directed products of hormone-treated and untreated tissue were the 35,000-dalton polypeptide(s) and the polypeptide earlier identified as α-amylase. The increased amount of the latter was detectable on the original fluorographs within 2 h (Fig. 2b) and was quite apparent by 4 h (Fig. 2d) and in all examples of the translation products of RNA from hormone-treated tissue thereafter (Fig. 2f, h and j).

The time course of the appearance of mRNA for α-amylase was estimated by calculating the percentage contribution made by α-amylase to total cell-free protein synthesis. Autoradiographs were prepared by exposing X-ray

film to the dried gels, similar to those in Fig. 2, for different times and the profiles were scanned by microdensitometry³⁵. α -Amylase made an increasing contribution to protein synthesis when the *in vitro* system was programmed with RNA extracted from tissue incubated with hormone for increasing periods up to 12 h (Fig. 3a). GA_3 treatment for 16 h did not stimulate further the production of template RNA for α -amylase (Fig. 3a). The *in vivo* production of total α -amylase in response to the hormone is shown in Fig. 3b plotted as the rate of α -amylase synthesis rather than as total enzyme level. There is a positive correlation between the rate of α -amylase synthesis *in vivo* and the level of mRNA for α -amylase as assayed by the cell-free system. Investigation of the stimulation of ovalbumin synthesis in chick oviduct by oestrogen has shown that the level of mRNA for ovalbumin increases in response to the hormone^{33,36,37}, and that the rate of synthesis of the oviduct protein is controlled chiefly by the concentration of cellular mRNA³⁸.

Our data show that GA_3 increases the level of translatable mRNA for α -amylase, but we cannot distinguish between the following three mechanisms whereby the level might be increased: (1) a decreased rate of mRNA degradation; (2) enhancement of the translational capacity of mRNA for α -amylase through processing, activation or release of RNA from some inactive form (this possibility

Fig. 2 Fluorographs of SDS-polyacrylamide gel electrophoresis of the cell-free translation products of RNA isolated from aleurone layers at various times after hormone treatment. Total RNA was extracted from aleurone layers (as described in the legend to Fig. 1) after 2, 4, 8, 12 and 16 h in buffer or buffer plus GA_3 and used to program the cell-free protein synthesising system. *a, c, e, g* and *i*, Translation products of total RNA from aleurone layers incubated without GA_3 for 2, 4, 8, 12 and 16 h respectively; *b, d, f, h* and *j*, translation products of total RNA from aleurone layers incubated with 10^{-6} M GA_3 for 2, 4, 8, 12 and 16 h respectively. The arrow denotes position of α -amylase and the numbers refer to molecular weight markers ($\times 10^{-3}$).

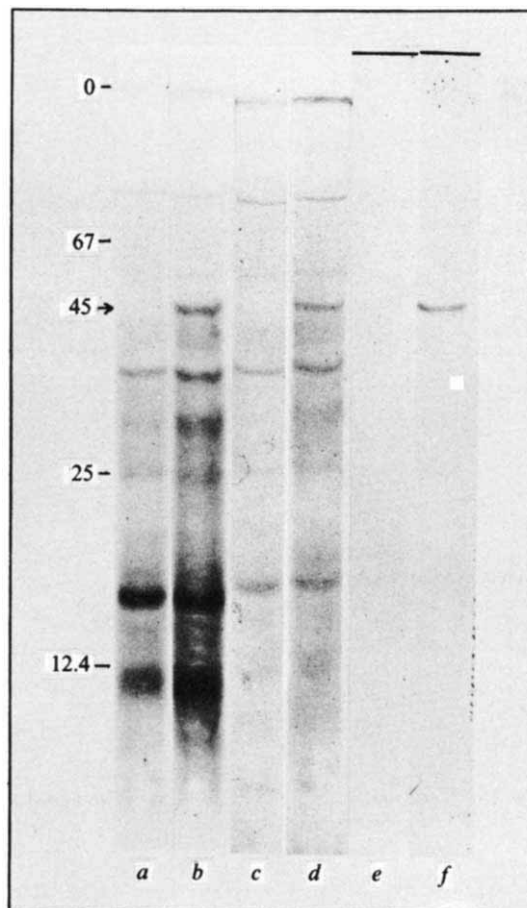
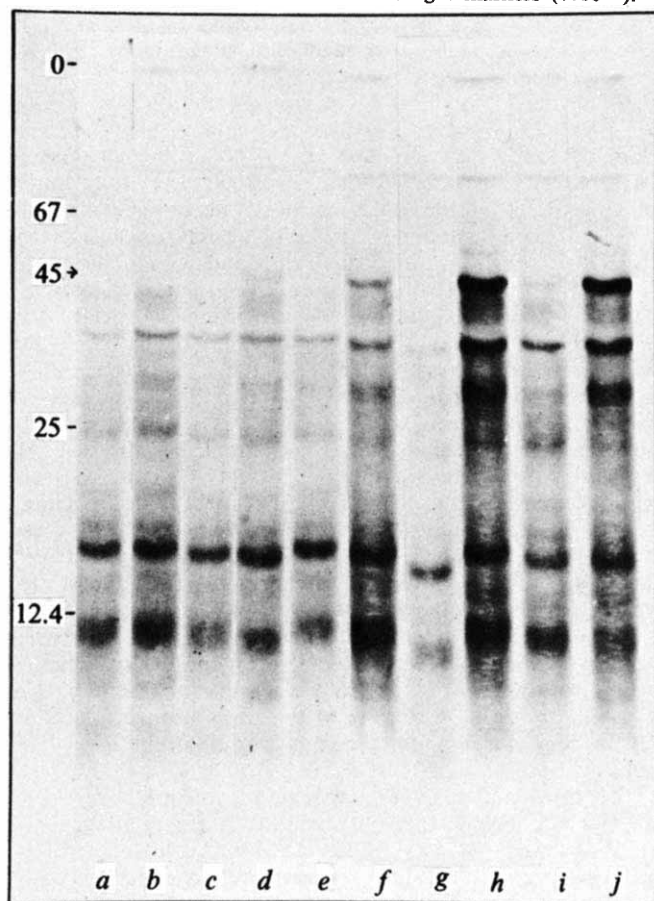


Fig. 3 The increase with time in the level of translatable mRNA for α -amylase and the increase in the rate of synthesis of the enzyme *in vivo* in response to GA_3 treatment. *a*, α -Amylase synthesised by the cell-free system was estimated from densitometric scans of autoradiographs of gels which had been in contact with X-ray film for different times. The gels were scanned using a Joyce-Loebl microdensitometer and the contribution made by the α -amylase peak to total protein synthesised was calculated by weight³⁵. *b*, The rate of α -amylase synthesis *in vivo* was calculated as the increment in enzyme level (units of α -amylase) during successive 2-h intervals following hormone treatment. Total α -amylase activity was assayed using replicates of 10 aleurone layers as described⁴. U, Units of α -amylase.

is relatively unlikely since total deproteinised RNA was used, eliminating the possibility that mRNP or other protein-masked forms of RNA were involved); and (3) synthesis of new mRNA molecules. Our results taken together with the fact that GA_3 stimulated poly(A)-RNA synthesis^{18,20}, although not eliminating an effect on degradation, provide correlative evidence that GA_3 promotes the synthesis of mRNA for α -amylase.

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T. J. V. HIGGINS
J. A. ZWAR
J. V. JACOBSEN

Division of Plant Industry, CSIRO,
PO Box 1600,
Canberra City, ACT 2601, Australia

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¹ Filner, P., and Varner, J. E., *Proc. natn. Acad. Sci. U.S.A.*, **58**, 1520 (1967).
² Varner, J. E., *Pl. Physiol.*, **39**, 413 (1964).

- ³ Varner, J. E., and Chandra, G. R., *Proc. natn. Acad. Sci. U.S.A.*, **52**, 100 (1964).
⁴ Chrispeels, M. J., and Varner, J. E., *Pl. Physiol.*, **Lancaster**, **42**, 398 (1967).
⁵ Goodwin, P. B., and Carr, D. J., *J. exp. Bot.*, **23**, 1 (1972).
⁶ Goodwin, P. B., and Carr, D. J., *Cytobios*, **2**, 165 (1970).
⁷ Goodwin, P. B., and Carr, D. J., *J. exp. Bot.*, **23**, 8 (1972).
⁸ Goodwin, P. B., *Plant Growth Substances*, 616 (Hirokawa, Tokyo, 1973).
⁹ Chandra, G. R., and Varner, J. E., *Biochim. biophys. Acta*, **108**, 583 (1965).
¹⁰ Varner, J. E., Chandra, G. R., and Chrispeels, M. J., *J. cell. comp. Physiol.*, **66**, Suppl. 1, 55 (1965).
¹¹ Zwar, J. A., and Jacobsen, J. V., *Pl. Physiol.*, **Lancaster**, **49**, 1000 (1972).
¹² Goodwin, P. B., and Carr, D. J., *Planta*, **106**, 1 (1972).
¹³ Evins, W. H., *Biochemistry*, **10**, 4295 (1971).
¹⁴ Johnson, K. D., and Kende, H., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2674 (1971).
¹⁵ Evins, W. H., and Varner, J. E., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1031 (1971).
¹⁶ Koehler, D. E., and Varner, J. E., *Pl. Physiol.*, **Lancaster**, **52**, 208 (1973).
¹⁷ Firn, R. D., and Kende, H., *Pl. Physiol.*, **Lancaster**, **54**, 911 (1974).
¹⁸ Jacobsen, J. V., and Zwar, J. A., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3290 (1974).
¹⁹ Jacobsen, J. V., and Zwar, J. A., *Aust. J. Pl. Physiol.*, **1**, 343 (1974).
²⁰ Ho, D. T. H., and Varner, J. E., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4783 (1974).
²¹ Roberts, B. E., and Paterson, B. M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2330 (1974).
²² Partington, G. A., Kemp, D. J., and Rogers, G. E., *Nature new Biol.*, **246**, 33 (1973).
²³ Laemmli, U. K., and Favre, M., *J. molec. Biol.*, **80**, 575 (1973).
²⁴ Higgins, T. J. V., Goodwin, P. B., and Whitfield, P. R., *Virology* (in the press).
²⁵ Click, R. E., and Hackett, D. P., *Biochim. biophys. Acta*, **129**, 74 (1966).
²⁶ Bonner, W. M., and Laskey, R. A., *Eur. J. Biochem.*, **46**, 83 (1974).
²⁷ Ralph, R. K., and Bellamy, A. R., *Biochim. biophys. Acta*, **87**, 9 (1963).
²⁸ Higgins, T. J. V., Mercer, J. F. B., and Goodwin, P. B., *Nature new Biol.*, **246**, 68 (1973).
²⁹ Verma, D. P. S., Nash, D. T., and Schulman, H. M., *Nature*, **251**, 74 (1974).
³⁰ Verma, D. P. S., MacLachlan, G. A., Byrne, H., and Ewings, D., *J. biol. Chem.*, **250**, 1019 (1975).
³¹ Jacobsen, J. V., Scandalios, J. G., and Varner, J. E., *Pl. Physiol.*, **Lancaster**, **45**, 367 (1970).
³² Jacobsen, J. V., and Knox, R. B., *Planta*, **112**, 213 (1973).
³³ Rhoads, R. E., McKnight, G. S., and Schimke, R. T., *J. biol. Chem.*, **248**, 2035 (1973).
³⁴ Milstein, C., Brownlee, G. G., Harrison, T. M., and Mathews, M. B., *Nature*, **239**, 117 (1972).
³⁵ Adesnik, M., in *Methods in Virology* (edit. by Maramorosch, K., and Koprowski, H.), **125** (Academic, New York, 1971).
³⁶ Chan, L., Means, A. R., and O'Malley, B. W., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1870 (1973).
³⁷ Palmiter, R. D., *J. biol. Chem.*, **248**, 8260 (1973).
³⁸ Palmiter, R. D., *Cell*, **4**, 189 (1975).

Short-chain fatty acids as inhibitors of gibberellin-induced amylolysis in barley endosperm

ABSCISIC acid (ABA) is the only plant growth substance so far reported^{1,2} to inhibit gibberellin(GA)-induced amylolysis in barley half seeds or α -amylase production in barley aleurone layers: the former physiological response has been used as a bioassay for ABA^{3,4}. But the isolation procedures normally used to extract ABA from plant tissue also extract certain short-chain fatty acids⁵. We now present

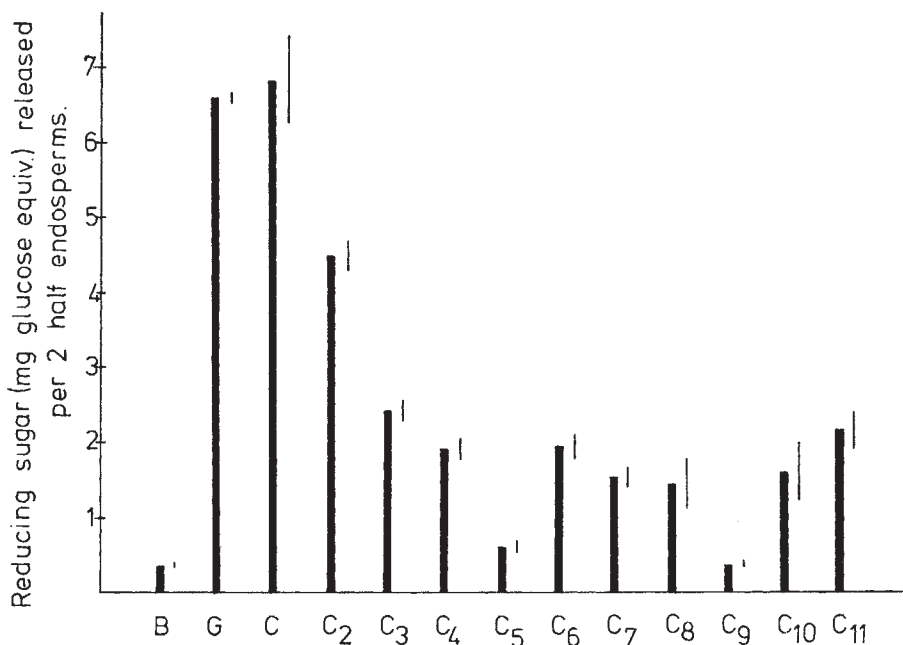
evidence that these naturally occurring short-chain acids⁶ are powerful inhibitors of GA-induced amylolysis in barley endosperm.

Embryo-free half seeds were prepared⁶ from husked barley (cultivar Ymer) and GA-induced starch hydrolysis was assayed by measuring^{7,8} the amount of reducing sugar released into the medium (1.05 ml) by pairs of half endosperms incubated in the presence and absence of GA (80% GA₃ 20% GA₁; 7.2×10^{-7} M) (B and G in Fig. 1). Buffering of the GA solution to pH 4.8 with citrate (10^{-3} M) did not alter the response to GA (C in Fig. 1). The effect of straight-chain carboxylic acids was assessed by replacing the citrate buffer with acetate (C₂), propionate (C₃), *n*-butanoate (C₄), *n*-pentanoate (C₅) *n*-undecanoate (C₁₁) buffers⁹ (10^{-3} M, pH 4.8) and the results are shown in Fig. 1. All these acids inhibit amylolysis to some extent; even acetate causes a 30% reduction in sugar release—a phenomenon first noted by Paleg¹⁰. The exceptional efficacy of pentanoic (C₅) and nonanoic (C₉) acids in overcoming the effect of GA is superimposed on a general increase in inhibition from C₁ to C₉ with a subsequent decrease at longer chain lengths.

Dose-response data for ABA and the four most inhibitory fatty acids (C₅, C₇, C₈ and C₉) are presented in Fig. 2. Clearly nonanoic acid (C₉) is the most effective of the fatty acids but the most striking feature of Fig. 2 is the difference between the dose-response curves for ABA on the one hand and the fatty acids on the other. Inhibition by ABA is total at 10^{-4} M, virtually zero at 10^{-8} M and at intermediate concentrations the response is proportional to the logarithm of the concentration. On the other hand, inhibition by fatty acids (for example, C₅ and C₉ in Fig. 2) is total at 10^{-3} M but nonexistent at 10^{-5} M. Stated differently, inhibition by ABA is a finely graded phenomenon, infinitely variable over a 10,000-fold concentration range whereas inhibition by fatty acids is more akin to a threshold effect. We have detected nonanoic acid in ungerminated barley seed and if such fatty acids are true plant growth substances—and evidence is accumulating rapidly that they are—the threshold effect could be important in the regulation of "all or nothing" phenomena such as germination or release from dormancy.

In Glasgow and in our laboratories in Stirling, reports are being prepared of positive correlations between fatty

Fig. 1 Influence of normal, short-chain fatty acids on GA-induced amylolysis in barley endosperm. The vertical bars represent the amount ($\pm$ s.e.m.) of reducing sugar released into the incubation medium by embryo-free half endosperms incubated in pairs (four replicates) for 42 h at 26 °C in the presence of water alone (B); 4.2×10^{-7} M gibberellin in water (G); 4.2×10^{-7} M gibberellin in 10^{-3} M citrate buffer pH 4.8 (C); 4.7×10^{-7} M gibberellin in 10^{-3} M acetate (C₂), propionate (C₃), butanoate (C₄) . . . undecanoate (C₁₁) buffers, all at pH 4.8.



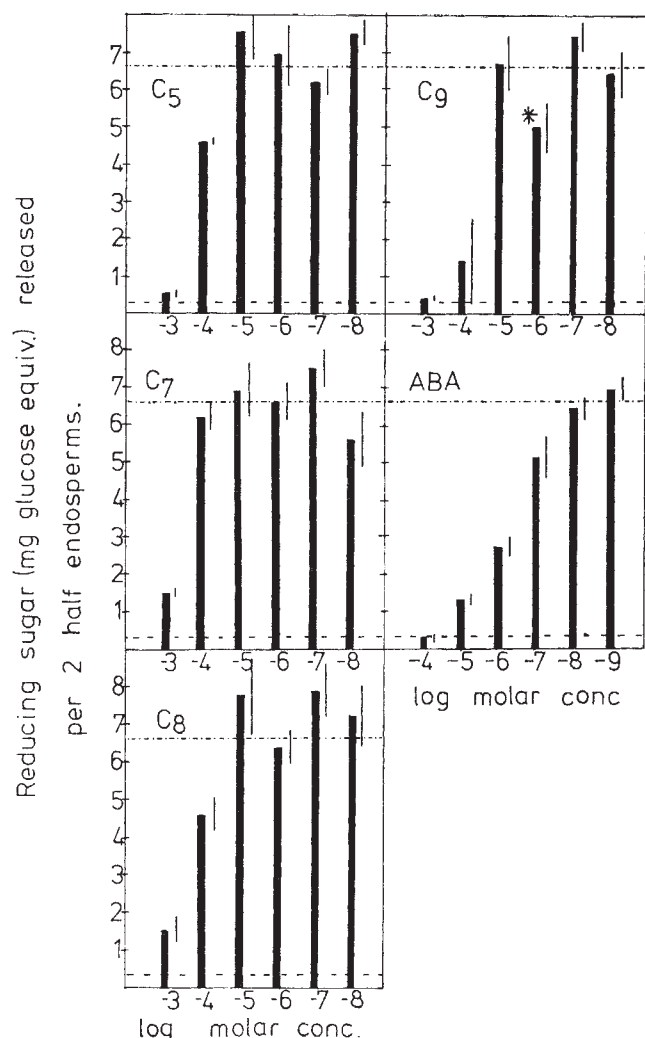


Fig. 2 Concentration dependence of fatty acid and ABA inhibition of GA-induced amylolysis in barley endosperm. Vertical bars represent reducing sugar released (see caption to Fig. 1) in presence of pentanoic (C_5), heptanoic (C_7), octanoic (C_8) and nonanoic (C_9) acids and ABA in the concentrations stated. All incubations were carried out at a constant GA concentration of 4.2×10^{-7} M, and pH 4.8. — — —, Mean blank value, that is, reducing sugar released in the absence of GA and inhibitors. ·····, Mean control value, that is, reducing sugar released in the presence of GA and absence of inhibitor. *Not significantly different from control value at $P < 0.10$.

acid levels and depth of dormancy in oat; aleurone layer activity in fenugreek seed; the mechanism of stomatal functioning, and leaf abscission and ethylene production. The *in vitro* inhibition of amylolysis described here represents yet another phenomenon which may have considerable physiological relevance.

DAVID C. BULLER
WILLIAM PARKER

Department of Chemistry,

J. S. GRANT REID

Department of Biochemistry,
University of Stirling,
Stirling FK9 4LA, UK

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- ¹ Addicott, F. T., Okhuma, K., Smith, O. E., and Thiessen, W. E., *Adv. Chem.*, **53**, 97–105 (1966).
- ² Chrispeels, M. J., and Varner, J. E., *Nature*, **212**, 1066–1067 (1966).
- ³ Goldsmith, E. E., and Monselise, S. P., *Pl. Physiol., Lancaster*, **43**, 113–116 (1968).
- ⁴ Sivori, E. M., Sonvico, V., and Fernandez, N. O., *Pl. Cell Physiol.*, **12**, 993–996 (1971).
- ⁵ Alam, M., Berrie, A. M. M., Don, R., Buller, D., and Parker, W., *Pl. Sci. Lett.* (in the press).
- ⁶ Coombe, B. G., Cohen, D., and Paleg, L. G., *Pl. Physiol., Lancaster*, **42**, 105–112 (1967).
- ⁷ Somogyi, M., *J. biol. Chem.*, **195**, 19–23 (1952).

- ⁸ Nelson, W., *J. biol. Chem.*, **153**, 375–380 (1944).
- ⁹ Weast, R. P. D., (edit.), *Handbook of Chemistry and Physics*, 54th ed. (Chemical Rubber Company, Cleveland, 1973).
- ¹⁰ Paleg, L. G., *Pl. Physiol., Lancaster*, **35**, 293–299 (1960).

DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA

INFECTION of fibroblasts by avian sarcoma virus (ASV) leads to neoplastic transformation of the host cell. Genetic analyses have implicated specific viral genes in the transforming process^{1–4}, and recent results suggest that a single viral gene is responsible⁴. Normal chicken cells contain DNA homologous to part of the ASV genome^{5–8}; moreover, embryonic fibroblasts from certain strains of chickens can produce low titres of infectious type C viruses either spontaneously⁹ or in response to various inducing agents¹⁰. None of the viruses obtained from normal chicken cells, however, can transform fibroblasts, and results with molecular hybridisation indicate that the nucleotide sequences responsible for transformation by ASV are not part of the genetic complement of the normal cell¹¹. We demonstrate here that the DNA of normal chicken cells contains nucleotide sequences closely related to at least a portion of the transforming gene(s) of ASV; in addition, we have found that similar sequences are widely distributed among DNA of avian species and that they have diverged roughly according to phylogenetic distances among the species. Our data are relevant to current hypotheses of the origin of the genomes of RNA tumour viruses¹² and the potential role of these genomes in oncogenesis¹³.

We have prepared radioactive DNA (cDNA_{sar}) complementary to nucleotide sequences which represent most or all of the viral gene(s) required for transformation of fibroblasts by ASV¹⁴. Our procedure to isolate cDNA_{sar} exploited the existence of deletion mutants of ASV which lack 10–20% of the viral genome (transformation defective, or td viruses)^{11,15–17}; results of genetic analyses indicate that the deleted nucleotide sequences include part or all of the gene(s) responsible for oncogenesis and cellular transformation^{4,14}. In our procedure the genome of the Prague-C strain (Pr-C) of ASV was transcribed into complementary DNA by endogenous RNA-directed DNA polymerase activity; we then used molecular hybridisation to select DNA specific for the region missing from the genome of the td deletion mutants. The preparation of cDNA_{sar} used was a virtually uniform transcript from about 16% of the Pr-C ASV genome¹⁴, a region equivalent in size to the entire deletion in the strain of td virus used in our experiments^{11,15–17}. Since the unit genome of ASV contains about 10,000 nucleotides^{18,19}, the genetic complexity of cDNA_{sar} is about 1,600 nucleotides, sufficient to represent an entire cistron. Nucleotide sequences homologous to cDNA_{sar} seem to be ubiquitous in the genomes of ASVs, but are not present in the genomes of avian leukosis viruses (including the endogenous chicken virus, RAV-0) or sarcoma-leukosis viruses from other species¹⁴.

DNAs from several avian species (chicken, turkey, quail, duck and emu) contain nucleotide sequences which can anneal with cDNA_{sar} (Fig. 1 and Table 1). In contrast, we detected no homology between cDNA_{sar} and DNA from mammals (mouse and calf thymus; Table 1). The kinetics of the reactions between cDNA_{sar} and DNAs from chicken, quail and duck (Fig. 1a, c and d) were similar to the kinetics for the reassociation of unique nucleotide sequences in avian DNAs (C_{ot} about $1,000 \text{ mol s}^{-1}$)²⁰; thus, nucleotide sequences homologous to cDNA_{sar} are present as single (or a few) copies in each haploid complement of the avian DNAs tested. This conclusion was substantiated by measuring in a single reaction

Table 1 Homology between cDNA_{sarc} and normal DNAs

Assay	Hybridisation conditions		Extent of reaction between cDNA _{sarc} and DNA from						
	[Na ⁺]	Temperature	Chicken	Quail	Turkey	Duck	Emu	Mouse	Calf
SI	0.9 M	68°	52%	46%	48%	45%	24%	<2%	<2%
HAP	0.9 M	68°					36%		<5%
HAP	1.5 M	59°					54%		

DNA was extracted from 10–11-d-old embryos of chickens, ducks and quails, 3-d-old mice (strain RIII), livers of adult turkeys, liver and heart of a 22-d-old emu, and calf thymus. Reaction mixtures containing denatured DNA (8 mg ml⁻¹) and ³H-cDNA_{sarc} (0.32 ng ml⁻¹, 7,000 c.p.m. ml⁻¹) in a final volume of 0.3 ml were incubated at either 59 or 68 °C for 48 h. Samples incubated at 59 °C were in 1.5 M NaCl (final *C*₀*t* = 40,000), those incubated at 68 °C were in 0.9 M NaCl (final *C*₀*t* = 32,000); all reactions also contained 0.001 M EDTA–0.02 M Tris-HCl, pH 7.4. Duplex formation was measured by either hydrolysis with SI nuclease²⁸ (in 0.3 M NaCl at 50 °C) or fractionation on hydroxyapatite (HAP) (samples adsorbed in 0.14 M sodium phosphate at 50 °C).

mixture the rates of reassociation between chicken DNA and both cDNA_{sarc} (labelled with ³H) and unique nucleotide sequences purified from chicken DNA (labelled with ¹⁴C) (Fig. 1b); the rates were similar for both labelled DNAs.

The extent of duplex formation with cDNA_{sarc} varied with the amount of chicken DNA used in the reactions

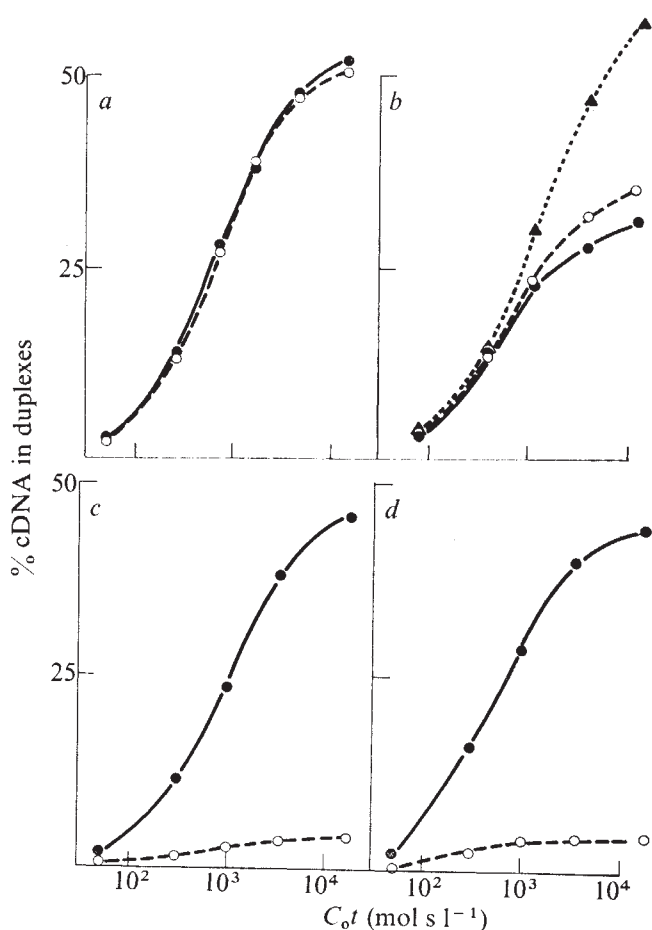


Fig. 1 Annealing of cDNA_{sarc} to normal avian DNAs. DNA was prepared from 10–11-d-old embryos⁷, denatured and incubated with ³H-cDNA_{sarc} and ³²P-cDNA_{B77} in 0.6 M NaCl–0.001 EDTA–0.02 M Tris-HCl, pH 7.4 at 68 °C for 0.1 to 32 h. Samples (0.1–0.2 ml) were withdrawn at different times and the extent of duplex formation with cDNA_{sarc} and cDNA_{B77} was measured by hydrolysis with the single-strand specific nuclease, SI (ref. 28). The preparation of cDNA_{sarc} and cDNA_{B77} has been described elsewhere¹⁴ and is outlined in the text. Unique sequence chicken DNA was prepared by reannealing denatured ¹⁴C-chicken DNA to a *C*₀*t* of 500; unique sequence DNA was eluted from a hydroxyapatite column at 60 °C in 0.14 M phosphate buffer²⁰. *a*, Chicken DNA (9 mg ml⁻¹) with ³H-cDNA_{sarc} (0.5 ng ml⁻¹, 10,000 c.p.m. ml⁻¹) (●) and ³²P-cDNA_{B77} (0.3 ng ml⁻¹, 10,000 c.p.m. ml⁻¹) (○). *b*, Chicken DNA (5 mg ml⁻¹) with ³H-cDNA_{sarc} (0.45 ng ml⁻¹, 9,000 c.p.m. ml⁻¹) (●); ³²P-cDNA_{B77} (0.5 ng ml⁻¹, 6,000 c.p.m. ml⁻¹) (○), and ¹⁴C-chicken unique sequence DNA (▲). *c*, Quail DNA (9 mg ml⁻¹) with ³H-cDNA_{sarc} and ³²P-cDNA_{B77} as in *a*. *d*, Duck DNA (9 mg ml⁻¹) with ³H-cDNA_{sarc} and ³²P-cDNA_{B77} as in *a*.

(Fig. 1a and b), as expected in reactions where identical labelled and unlabelled DNA strands are competing for unlabelled complementary strands, and the reactions were incomplete (about 50%) at the highest value of *C*₀*t* (Fig. 1a, c and d and Table 1). Nevertheless, we believe that most or all of the nucleotide sequences of cDNA_{sarc} are present in the DNA of quail since cDNA_{sarc} anneals completely with RNA from certain quail cells (unpublished results of ourselves and C. Moscovici). Moreover, the rates and extents of annealing between cDNA_{sarc} and DNA from chicken, duck and turkey are approximately the same as the rate and extent of annealing between cDNA_{sarc} and quail DNA (Table 1 and Fig. 1). Thus, it is likely that most or all of the nucleotide sequences of cDNA_{sarc} are present in the DNA of all these birds.

The extent of the reaction between cDNA_{sarc} and DNA from emu, a relatively primitive Australian bird, was limited (24%) when tested by hydrolysis with a single strand-specific nuclease (Table 1); the extent of the reaction was greater when analysed on hydroxyapatite, a less stringent procedure than the nuclease test (Table 1), and was further augmented when the annealings were performed in conditions which facilitate pairing of partially matched nucleotide sequences (1.5 M NaCl, 59 °C, ref. 21) (Table 1). These data indicate that the nucleotide sequences homologous to cDNA_{sarc} in emu DNA are substantially diverged from the homologous sequences in the other avian DNAs; we have obtained further data to sustain this conclusion by analysing the thermal stability of the duplexes formed between cDNA_{sarc} and various avian DNAs (see below, Table 2 and Fig. 2).

Avian DNAs were also tested with ³²P-labelled single-stranded DNA, complementary to the RNA genome of the B77 strain of ASV (cDNA_{B77}), synthesised with detergent-disrupted virions, and purified as described previously¹⁴. The virus used to prepare cDNA_{B77} consisted mainly of td variants (about 90% of the particles; unpublished observations). Consequently, DNA synthesised with the virus was deficient in nucleotide sequences homologous to cDNA_{sarc} and served principally to detect other portions of the ASV genome. A substantial fraction (about 50%) of cDNA_{B77} reacted with normal chicken DNA, but there was little or no reaction with quail, duck, turkey and emu DNAs (Fig. 1 and unpublished data); these results conform to previous reports^{20,22,23}. We conclude that the DNAs from widely divergent avian species all contain nucleotide sequences which are at least partially related to transforming gene(s) of ASV, whereas only chicken DNA has appreciable homology with the remainder of the ASV genome.

The relatedness of DNA sequences homologous to cDNA_{sarc} in different avian species was analysed by denaturing duplexes formed between cDNA_{sarc} and normal avian DNAs (Fig. 2 and Table 2). In addition, we denatured duplexes between cDNA_{sarc} and DNA from XC cells (rat cells transformed by Pr-C ASV) to test completely matched duplexes containing cDNA_{sarc} sequences. The mammalian DNAs we have tested (calf and mouse) contain no nucleotide sequences homologous to cDNA_{sarc} before in-

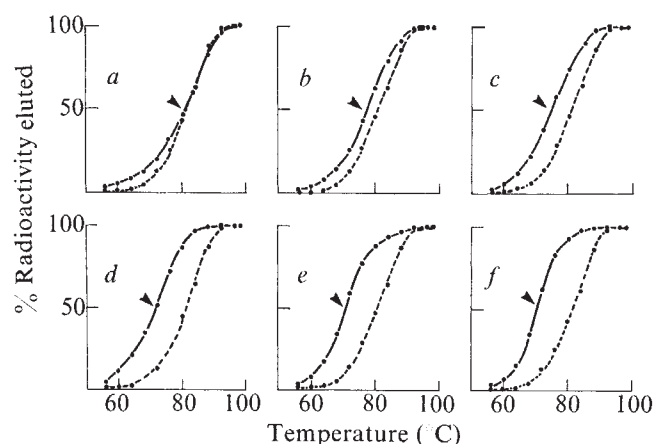


Fig. 2 Thermal denaturation of duplexes formed between cDNA_{sarc} and cellular DNAs. DNA was extracted from XC cells grown in culture, from 10–11-d-old embryos of chickens, ducks and quails, from the livers of adult turkeys, and from liver and heart of a 22-d-old emu. Denatured DNAs (0.5 mg) were incubated with ³H-cDNA_{sarc} (0.25 ng, 5,000 c.p.m.) in 0.1 ml of 0.9 M NaCl–0.001 M EDTA–0.02 M Tris-HCl, pH 7.4, for 100 h at 68°C, final $C_0t = 40,000$. In a separate reaction, ³²P-cDNA_{B77} (2.5 ng, 100,000 c.p.m.) was annealed with 7 mg of denatured chicken DNA (final $C_0t = 80,000$). Samples (0.4 mg of chicken DNA and 6,000 c.p.m. of cDNA_{B77}) of this reaction were added to the DNAs which had been annealed with cDNA_{sarc}. The mixtures were then passed through a column of hydroxyapatite (3 ml packed vol) in 0.12 M sodium phosphate, pH 6.8, at 56°C; in these conditions, stable duplexes bind to the hydroxyapatite, whereas single-stranded DNA does not. The columns were then washed continuously with 0.12 M sodium phosphate (0.4 ml min⁻¹) while the temperature of the column was raised in increments of 4°C every 10 min. Fractions (4 ml) were collected and analysed for acid-precipitable radioactivity. ●—●, Duplexes with cDNA_{sarc}; ○—○, duplexes with cDNA_{B77} and chicken DNA (internal standard). a, Provirus (XC); b, chicken; c, quail; d, turkey; e, duck; f, emu.

fection by ASV; consequently, the DNA homologous to cDNA_{sarc} in rat cells after infection presumably represents nucleotide sequences contained entirely in recently integrated provirus for ASV rather than related sequences present in the DNA of uninfected cells. Denaturation was standardised internally by adding duplexes formed in a separate annealing reaction between normal chicken DNA and ³²P-labelled cDNA_{B77}; these duplexes denatured with a T_m of $81 \pm 1^\circ\text{C}$.

Duplexes between cDNA_{sarc} and DNA from XC cells had the highest T_m (81°C ; Table 2), as expected for completely matched complementary nucleotide sequences. Duplexes with chicken DNA were slightly less stable ($T_m = 77^\circ\text{C}$); the nucleotide sequences of cDNA_{sarc} in the ASV genome are apparently diverged from the homologous sequences in the avian host. The reduction in the T_m is consistent with about 3% mismatching of bases²⁴. The stability of duplexes with other avian DNAs ($T_m = 70\text{--}74^\circ\text{C}$) decreased roughly in accord with phylogenetic distances among the species

tested (Table 2); the T_m s suggest 5–8% mismatching of base pairs²⁴. We made no effort in these tests to obtain maximum duplex formation with cDNA_{sarc}, and the reactions with quail, turkey, duck and emu were relatively limited in extent (15–37%). In other experiments where at least 50% of cDNA_{sarc} was annealed into duplexes with chicken, quail and duck DNAs, we observed T_m s similar to those given in Table 2. cDNA_{sarc} seems to represent nucleotide sequences which arose and diverged during the course of avian speciation.

We have shown previously that the nucleotide sequences of cDNA_{sarc} include genetic information required for transformation of fibroblasts by ASV¹⁴; the data reported here indicate that similar or partially related information is present in the genome of the normal chicken cell and widely distributed among the avian species. We suggest that part or all of the transforming gene(s) of ASV was derived from the chicken genome or a species closely related to chicken, either by a process akin to transduction²⁵ or by other events, including recombination, which are alleged to have generated type C viruses from normal cellular genes¹². If the entire ASV genome originated from cellular genes¹², then nucleotide sequences in the transforming gene(s) have been conserved relative to other viral genes, because the nucleotide sequences of cDNA_{sarc} are the only portion of the ASV genome which we can detect in DNA from birds other than chickens. We cannot exclude, however, the possibility that viral genes other than those represented by cDNA_{sarc} have been introduced into the germ line of chickens after speciation. The sequences homologous to cDNA_{sarc} in the genome of ASV are slightly diverged from the analogous sequences in chicken genome; this could be the consequence of either the process which generated viral genes from cellular genes¹² or mutations during the course of repeated viral propagation.

Others have reported evidence concerning the genetic divergence during possible spread of unidentified portions of type C viral genomes during evolution^{26,27} and the apparent transduction of cellular nucleotide sequences by type C viruses²⁵, but our study provides the first data on the origins of a genetically identified set of viral nucleotide sequences. It should be possible to carry out similar studies for at least one other gene of ASV (the gene coding for the type-specific glycoprotein), using techniques similar to those reported here.

Neiman and his colleagues have reported that the hybridisation of ASV 70S RNA to normal chicken DNA was competed completely by RNA from non-transforming viruses¹¹. They therefore concluded that the genes responsible for transformation by ASV were present in the avian genome only after viral infection. We cannot explain the discrepancy between their results and ours.

We anticipate that cellular DNA homologous to cDNA_{sarc} serves some function which accounts for its conservation during avian speciation. The nucleotide sequences which anneal with cDNA_{sarc} are part of the

Table 2 Thermal stabilities of duplexes between cDNA_{sarc} and normal DNAs

DNA	%cDNA _{sarc} in duplexes	T_m	ΔT_m	Phylogenetic distance from chicken (Myr)
Provirus (XC)	56	81	0	
Chicken	52	77	– 4	0
Quail	37	74	– 7	35–40
Turkey	30	72	– 9	40
Duck	16	71	– 10	80
Emu	15	70	– 11	100

Denatured DNAs (0.5 mg) were annealed with ³H-cDNA_{sarc} (0.25 ng, 5,000 c.p.m.), adsorbed to hydroxyapatite in 0.12 M sodium phosphate at 56°C, and denatured with a thermal gradient, all as described for Fig. 2. Duplexes between ³²P-cDNA_{B77} and normal chicken DNA, included in each analysis as an internal standard, denatured with $T_m = 81 \pm 1^\circ\text{C}$. The estimates of phylogenetic distance, deduced from fossil records and antigenic relationships among proteins²⁹, were provided by Professor Allan Wilson (personal communication).

unique fraction of cellular DNA and could represent either structural or regulatory genes. But the function of those sequences is unknown. We are testing the possibilities that they are involved in the normal regulation of cell growth and development or in the transformation of cell behaviour by physical, chemical or viral agents.

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D. STEHELIN*
H. E. VARMUS
J. M. BISHOP

Department of Microbiology,
University of California,
San Francisco, California 94143

P. K. VOGT

Department of Microbiology,
University of California,
Los Angeles, California 90033

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*Present address: IRSC BP8, 94800-Villejuif, France.

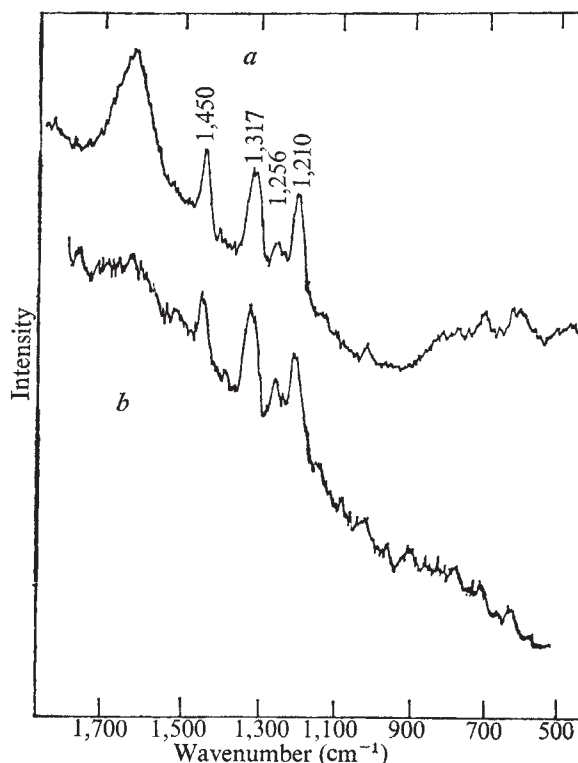
- ¹ Martin, G. S., *Nature*, 227, 1021-1023 (1970).
- ² Kawai, S., and Hanafusa, H., *Virology*, 46, 470-479 (1971).
- ³ Bader, J. P., *J. Virol.*, 10, 267-276 (1972).
- ⁴ Wyke, J. A., Bell, J. G., and Beaman, J. A., *Cold Spring Harb. Symp. quant. Biol.*, 39, 897-905 (1974).
- ⁵ Baluda, M. A., *Proc. natn. Acad. Sci. U.S.A.*, 69, 576-580 (1972).
- ⁶ Rosenthal, P. N., Robinson, H. L., Robinson, W. S., Hanafusa, T., and Hanafusa, H., *Proc. natn. Acad. Sci. U.S.A.*, 68, 2336-2340 (1971).
- ⁷ Varmus, H. E., Weiss, R. A., Friis, R. R., Levinson, W., and Bishop, J. M., *Proc. natn. Acad. Sci. U.S.A.*, 69, 20-24 (1972).
- ⁸ Wright, S. E., and Neiman, P. E., *Biochemistry*, 13, 1549-1554 (1974).
- ⁹ Vogt, P. K., and Friis, R. R., *Virology*, 43, 223-234 (1971).
- ¹⁰ Weiss, R. A., Friis, R. R., Katz, E., and Vogt, P. K., *Virology*, 46, 920-938 (1971).
- ¹¹ Neiman, P. E., Wright, S. E., McMillin, C., and MacDonnell, D., *J. Virol.*, 13, 837-846 (1974).
- ¹² Temin, H. M., *Cancer Res.*, 34, 2835-2841 (1974).
- ¹³ Huebner, R. J., and Todaro, G. J., *Proc. natn. Acad. Sci. U.S.A.*, 64, 1087-1094 (1969).
- ¹⁴ Stehelin, D., Guntaka, R. V., Varmus, H. E., and Bishop, J. M., *J. molec. Biol.* (in the press).
- ¹⁵ Duesberg, P. H., and Vogt, P. K., *Proc. natn. Acad. Sci. U.S.A.*, 67, 1673-1680 (1970).
- ¹⁶ Lai, M. M. C., Duesberg, P. H., Horst, J., and Vogt, P. K., *Proc. natn. Acad. Sci. U.S.A.*, 70, 2266-2270 (1973).
- ¹⁷ Duesberg, P. H., and Vogt, P. K., *J. Virol.*, 12, 594-599 (1973).
- ¹⁸ Beemon, K., Duesberg, P., and Vogt, P. K., *Proc. natn. Acad. Sci. U.S.A.*, 71, 4254-4258 (1974).
- ¹⁹ Billeter, M. A., Parsons, J. T., and Coffin, J. M., *Proc. natn. Acad. Sci. U.S.A.*, 71, 3560-3564 (1974).
- ²⁰ Varmus, H. E., Heasley, S., and Bishop, J. M., *J. Virol.*, 14, 895-903 (1974).
- ²¹ Rice, N., and Paul, P., *Yb. Carnegie Instn. Wash.*, 71, 262-264 (1972).
- ²² Tereba, A., Skoog, L., and Vogt, P. K., *Virology*, 65, 524-534 (1975).
- ²³ Kang, C. Y., and Temin, H. M., *J. Virol.*, 14, 1179-1188 (1974).
- ²⁴ Ullman, J. S., and McCarthy, B. J., *Biochim. biophys. Acta*, 294, 405-415 (1973).
- ²⁵ Scolnick, E. M., Rands, E., Williams, D., and Parks, W. P., *J. Virol.*, 12, 458-463 (1973).
- ²⁶ Benveniste, R. E., and Todaro, G. J., *Proc. natn. Acad. Sci. U.S.A.*, 71, 4513-4518 (1974).
- ²⁷ Benveniste, R. E., and Todaro, G. J., *Nature*, 252, 456-459 (1974).
- ²⁸ Leong, J. A., et al., *J. Virol.*, 9, 891-902 (1972).
- ²⁹ Praeger, E. M., Brush, A. H., Nolan, R. A., Nakanishi, M., and Wilson, A. C., *J. molec. Evol.*, 3, 243 (1974).

laser beams of visible light¹⁻⁶. They all show a number of Raman lines characteristic of the four common bases, namely, adenine, uracil, guanine, and cytosine. For such observations concentrated solutions (usually about 5 %) or considerable amounts (10 mg) of tRNA are required. Some of the minor constituent nucleosides in tRNAs have strong absorption bands in the 300-350-nm region, whereas the common bases have them at 260 nm. Using the correct ultraviolet laser, therefore, one would expect to observe resonance Raman effects of minor nucleoside bases. Such effects would be valuable, first, because they would provide information on one particular base residue of the 70-80 bases in tRNA and second, because this could be done with much smaller amount samples of tRNA this usual.

We have observed a Raman spectrum of formylmethionine tRNA of *Escherichia coli* with the 363.8-nm beam of an argon ion laser (Coherent Radiation 52 GA). The power of the laser beam was 5 mW at the sample. The tRNA concentration of the sample solution was only 0.3 %, in cacodylate buffer, pH 7.2. The total volume of the sample solution used was 0.2 ml, contained in a cylindrical cell of silica glass. A JRS-U1 Raman spectrophotometer (Japan Electron Optics) was used. The recorded curve is shown in Fig. 1a. The curve can be reproduced more than ten times from one sample solution. The aminoacylation activity of the tRNA remained undegraded after 5 h irradiation with the ultraviolet laser beam. When the wavelength of the laser beam was changed from 363.8 to 351.1 nm, almost the same Raman spectrum was observed.

All of the observed Raman lines are attributable to the 4-thiouridine residue (at position 8) subjected to photochemical modification (probably cross linking^{7,8} with the cytidine residue at position 13 of the tRNA). This can be concluded because, first, at that concentration and with that laser power, on the basis of the available data, none of the Raman lines of the common base residues can be expected to appear. Moreover, none of the

Fig. 1 Raman spectra excited by the 3,638 Å beam of an argon ion laser. a, tRNA^{Met} from *E. coli*; concentration, 0.3 %; solvent, 10⁻¹ M NaCl + 10⁻² M Na-cacodylate (pH 7.2) + 10⁻³ M MgCl₂. b, tRNA^{Gly} from *E. coli*; concentration, 0.3 %; solvent as in a.



Raman spectra of transfer RNAs with ultraviolet lasers

We suggest here a new use for Raman spectroscopy in structural studies of nucleic acids. We demonstrate that an enhanced vibrational Raman spectrum of a certain limited portion of an amino acid transfer RNA (tRNA) macromolecule can be observed, if a proper ultraviolet laser beam is chosen for excitation.

Until now, Raman spectra of tRNAs have been observed with

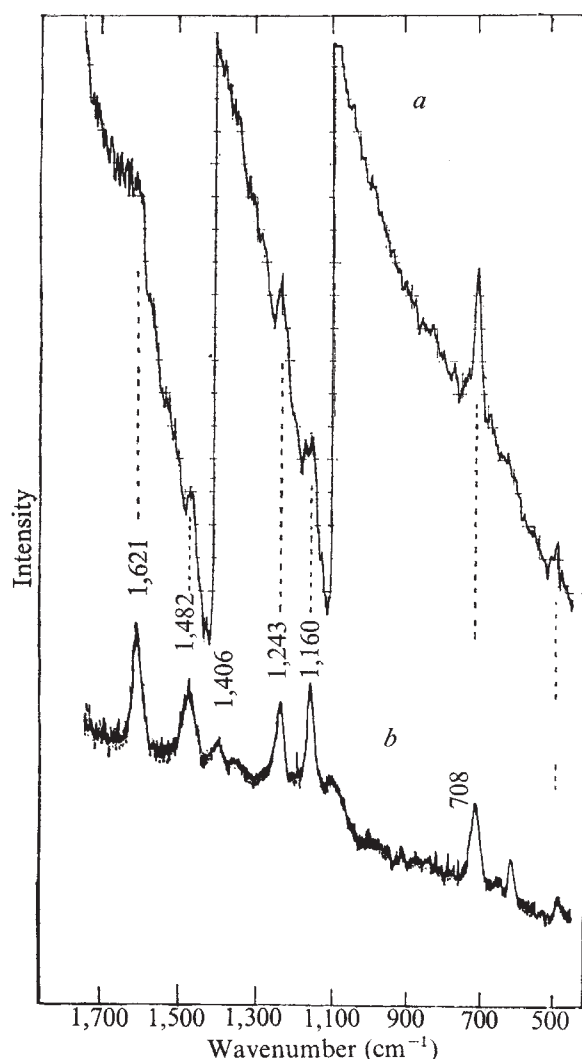


Fig. 2 Raman spectra excited by the 3,638 Å beam of an argon ion laser. *a*, tRNA^{Tyr} (2) from *E. coli*; concentration, 1%; solvent, 10^{-1} M NaCl + 10^{-2} M Na-cacodylate (pH 7.2), + 10^{-3} M MgCl₂. *b*, 4-Thiouridine; concentration, 10^{-4} M; solvent, 10^{-2} M Na-cacodylate, pH 7.2; observed with a rotating cell.

observed lines has the same Raman frequency as the common base residues. Second, 4-thiouridine has a strong absorption band at 330 nm, and is the only base (in this tRNA) known to have an absorption band in the 300–350-nm region. Even when 4-thiouridine forms a cross linkage with cytidine, it shows an absorption band of nearly equal intensity at 330 nm (ref. 8).

Third, none of the Raman lines in question has the Raman frequencies (1,621, 1,482, 1,243, 1,160, and 708 cm⁻¹) of 4-thiouridine itself (see Fig. 2*b*). The 708 cm⁻¹ line of 4-thiouridine can be assigned to the C=S stretching vibration. In the Raman spectrum now in question, on the other hand, no line assignable to the C=S stretching vibration is found. Thus, a structure lacking a C=S bond is suggested.

Fourth, *E. coli* tyrosine tRNA(2), which has 4-thiouridine residues (see, for example, ref. 9), (two at positions 8 and 9), but no cytidine residue¹⁰ at position 13 shows the Raman lines of 4-thiouridine. The sample is fluorescent, but on the fluorescence background all of the five stronger Raman lines of 4-thiouridine can be identified (Fig. 2*a*).

Fifth, *E. coli* glutamic acid tRNA(2), which has no 4-thiouridine residue¹¹, has been found to give no Raman lines in these conditions. Sixth, when a bulk tRNA sample of *E. coli* is placed in a rotating cell¹² the Raman lines of 4-thiouridine do

appear, but after a few runs of the spectrum with the ultraviolet laser they are gradually replaced by the Raman lines discussed.

The photochemical modification (probably the S⁴U–C linking) is considered to be caused in this case by the laser beam itself. When the rotating cell is used, the chance of the laser photon hitting the intact tRNA molecule would increase, so that the Raman lines of 4-thiouridine free from the cross linkage appear.

Most of the *E. coli* tRNAs have one or two 4-thiouridine residues. Using a similar Raman spectroscopic examination we found that tRNA^{Gly} should have the 4-thiouridine residue, and that this must be involved in a similar photochemical modification (probably the 4-thiouridine-cytidine cross linkage) (see Fig. 1*b*). (Although no 4-thiouridine has been reported¹³ in the primary sequences of tRNA^{Gly}, chemical analysis have shown¹⁴ that this tRNA contains 1 mol of 4-thiouridine.) Also for tRNA^{Met} (ref. 15) of *Thermus thermophilus*, an extremely thermophilic bacterium¹⁶, a similar Raman spectrum attributable to the 4-thiouridine-cytidine cross linkage has been observed.

The ultraviolet laser would also be useful in obtaining information for other chromophore groups in tRNAs, for example, the Y base in yeast tRNA^{Phe} (ref. 17).

We thank Professor H. Hayatsu, University of Tokyo, for providing the sample of 4-thiouridine.

YOSHIFUMI NISHIMURA
AKIKO Y. HIRAKAWA
MASAMICHI TSUBOI

Faculty of Pharmaceutical Sciences,
University of Tokyo,
Hongo, Bunkyo-ku, Tokyo

SUSUMU NISHIMURA

National Cancer Center,
Research Institute,
Chuo-ku, Tokyo, Japan

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- 1 Tsuboi, M., Takahashi, S., Muraishi, S., Kajiura, T., and Nishimura, S., *Science*, **174**, 1142–1144 (1971).
- 2 Small, E. W., and Peticolas, W. L., *Biopolymers*, **10**, 1377–1416 (1971).
- 3 Thomas, G. J., Medeiros, G. C., and Hartman, K. A., *Biochim. biophys. Acta*, **277**, 71–79 (1972).
- 4 Thomas, G. J., Chen, M. C., and Hartman, K. A., *Biochim. biophys. Acta*, **324**, 37–49 (1973).
- 5 Chen, M. C., and Thomas, G. J., *Biopolymers*, **13**, 615–626 (1974).
- 6 Dobek, A., Patkowski, A., Labuda, D., and Augustyniak, J., *J. Raman Spectrosc.*, **3**, 45–54 (1975).
- 7 Yaniv, M., Favre, A., and Barrell, B. G., *Nature*, **223**, 1331–1335 (1969).
- 8 Bergstrom, D. E., and Leonard, N. J., *Biochemistry*, **11**, 1–8 (1972).
- 9 RajBhandary, U. L., et al., *Fedn Proc.*, **28**, 409–409 (1969).
- 10 Goodman, H. M., Abelson, J. N., Landy, A., Zadrazil, S., and Smith, J. D., *Eur. J. Biochem.*, **13**, 461–483 (1970).
- 11 Ohashi, Z., Harada, F., and Nishimura, S., *FEBS Lett.*, **20**, 239–241 (1972).
- 12 Kiefer, W., and Bernstein, H. J., *J. appl. Spectrosc.*, **25**, 500–501 (1971).
- 13 Squires, C., and Carbon, J., *Nature new Biol.*, **233**, 274–277 (1971).
- 14 Nishimura, S., *Progr. nucl. Acid Res. molec. Biol.*, **12**, 49–85 (1972).
- 15 Oshima, T., Sakaki, Y., Wakayama, N., Watanabe, K., Ohashi, Z., and Nishimura, S., *Experientia* (in the press).
- 16 Oshima, T., and Imahori, K., *Int. J. system. Bacteriol.*, **24**, 102–112 (1974).
- 17 RajBhandary, U. L., and Chang, S. H., *J. biol. Chem.*, **243**, 598–608 (1968).

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The **Index** for 1975 is now available, price £2.25. Copies of the 1974 index are still on sale, price £3.00. **Binders** for the journal are also available at £2.30 each, £6.25 for three (a year of *Nature* fits into three binders).

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reviews

New Zealand archipelago

Malcolm Coe

At a time when the trend is towards publication of specialist volumes dealing with individual animal and plant groups, this broad approach* will be welcomed by the biologist who is anxious to obtain an overall assessment of an area's fauna. In using other volumes in this interesting series, I have always felt that much would have been gained by attempting to use the same format for each volume. One appreciates, however, the difficulty of persuading authors to write on anything other than their own particular specialist field. The comparative isolation of the New Zealand archipelago makes its study of particular interest to the biogeographer, for 80% of its flowering plants and 90% of its arthropods are endemic.

The first of 17 chapters sets out to describe the geological history of the archipelago as a background for the later account of the development and evolution of the main biota. In a concise account of an isolated flora and fauna, one is reminded of the drastic effect man has had on this environment when he first arrived there a 1,000 or so years ago and exterminated the moas, several species of Carinate birds and even a variety of previously prominent invertebrates. For those who still doubt the association of man with dramatic post-Pleistocene extinction it should make interesting reading. This account is followed by two chapters dealing with the climate and soils which form essential background reading on the description of vegetation which follows.

The story of man-induced changes in the biota is a continuing theme throughout the volume and considering how comparatively recent the changes are it is the more remarkable when we consider the influence that such features as the extermination of moas must have had on a vegetation for which they were the dominant large herbivore.

Although the terrestrial vertebrate fauna is only represented by 100 species, comprising 30 reptiles, 65 land birds

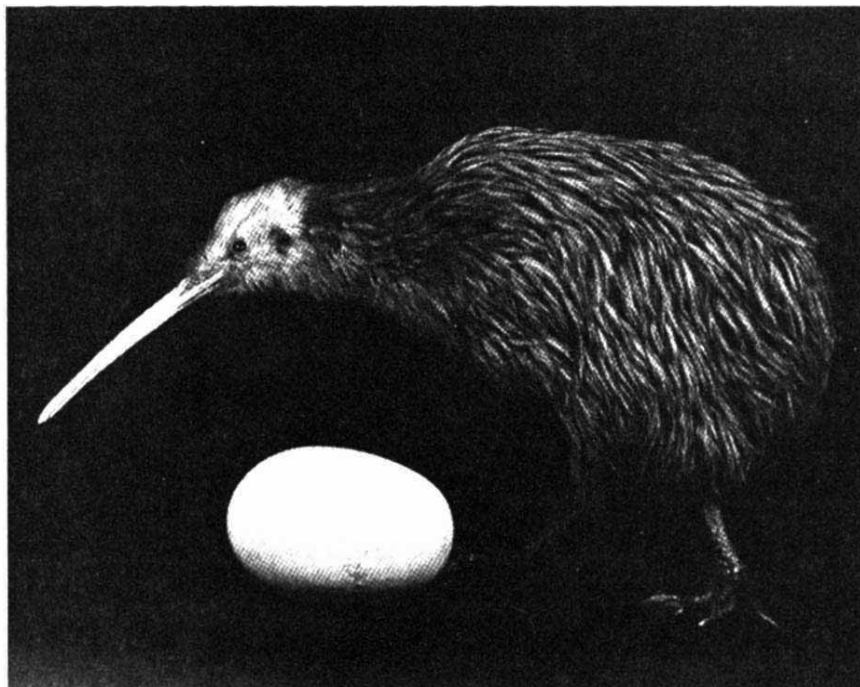


Photo: National Museum

North Island brown kiwi, *Apteryx a. mantelli*, female (440 g, 125 × 78.5 mm) and egg (400 ml)

and two mammals, this small number represents a fascinating illustration of adaptive radiation in isolation, of which probably the development of the terrestrial parrots is perhaps the most dramatic. Considering that a further 70 species of exotic birds and mammals have been introduced to New Zealand, it is surprising that even more elements of the endemic fauna have not become extinct. The fishes, in contrast to other vertebrate groups, comprise 8 families, none of which are endemic; and perhaps the greatest feature of interest is their ability to move between fresh and salt water, indicating no doubt the route by which freshwater habitats in New Zealand have been occupied. The kiwi and *Sphenodon* are justifiably given chapters of their own.

Aquatic environments are discussed in two chapters dealing with the marine benthic and freshwater biota, respectively. The greater degree of isolation experienced by the freshwater flora and fauna make them of somewhat greater biogeographical interest. Perhaps one of the

greatest features of interest that emerges when we examine these 'early' groups is that comparatively few of them are represented by endemic forms, illustrating the comparative geological youth of New Zealand.

The land molluscs, arachnids, and terrestrial and freshwater insects all provide valuable examples of radiation and speciation.

The final two chapters deal with the adaptation and change in Maori culture and the influence of man on the biota. I found these two chapters disappointing, for having learned a great deal about the flora and fauna of New Zealand, the great gap in our knowledge lies in considering the ecology of the early colonists in relation to the landscape change, which has perhaps been more dramatic in New Zealand than almost any other part of the Earth's surface.

It is easy to criticise the features either included in or excluded from this volume but the resulting work will be an important source book for all students of the biogeography of Australasia. □

**Biogeography and Ecology in New Zealand*. (Monographiae Biologicae, Volume 27). Edited by G. Kuschel. Pp. xvi + 689. (Junk, Hague, 1975.) 200 Dutch guilders.

Plasma proteins

The Plasma Proteins: Structure, Function and Genetic Control, Volume 1. Edited by Frank W. Putnam. Second Edition. Pp. xvi+481. (Academic: New York and London, October 1975.) \$37.50; £19.50.

THE appearance of volume 1 of the second edition of *The Plasma Proteins* is to be welcomed for several reasons. Most important is that it provides a survey of the multifarious developments which have occurred in the 15 years following the publication of the first edition. The mass of data now available finally decided the editor to subdivide the authorship so that a number of specialists are now responsible for particular chapters. As is stated in the introduction, however, this approach was adopted with some hesitation because of the danger that an integrated coverage might be lost. An initial attempt by the editor to write the whole of the second edition himself became impossible because "the rate of progress in plasma proteins outstripped my pen". Finally, in volume 1 only two chapters, constituting a general survey of the plasma proteins and a chapter on transferrin, have been written by the editor. The survey is valuable but, in view of the high price of the volume, a case can be made out that a wider readership would have been reached if this

survey were to have appeared separately.

The second reason for welcoming this book is that it is well up to date. Probably because of the desirability for rapid publication, a few minor matters were not tidied up—for example, on p279 appears a warning which was already given on p272 that the iron-saturated and iron-free forms of transferrin can only be separated on starch gel if the starch is iron free. Also both Glx and Glu appear as abbreviations for glutamic acid. Table 1 of Chapter 2 represents a useful updating of the previous list of properties of plasma proteins. It is unfortunate, however, that reference to the original papers from which the data has been taken is indirect by way of another chapter in volumes 1, 2 or 3.

Since the appearance of the first edition, patient work in many laboratories has brought to light the complete sequences of a number of plasma proteins. Thus it may be appropriate to enquire as to the return which has resulted so far from this immense investment of time and energy. A positive answer in respect of the H and L immunoglobulin chains, albumin and the α_1 acid glycoprotein may be given with confidence. As is explained in chapter 3, the division of the albumin molecule into domains and the structures associated with the binding sites are now known. For α_1 acid glycoprotein the outcome is even more satisfactory

since it is now apparent that this protein is the first single-chain plasma protein having some homology with two other plasma proteins: IgG and haptoglobin. These results suggest that the time points at which each of the currently existing plasma proteins branched off from their primitive precursors will become known. **A. H. Gordon**

Organometallics

Organometallic Derivatives of the Main Group Elements. (International Review of Science. Inorganic Chemistry Series Two, Vol. 4.) Edited by B. J. Aylett. Pp. 417. (Butterworth: London; University Park Press: Baltimore, Maryland, August 1975.) £13.45.

ORGANOMETALLIC chemists are well served by surveys intended to ease the task of keeping abreast of the flood of papers on the subject. The Annual Surveys, forming part of the *Journal of Organometallic Chemistry*, provide detailed coverage of derivatives of individual metals or groups of metals; the Specialist Periodical Reports of The Chemical Society cover the whole field annually; and the series of which this volume forms a part covers derivatives of the main group elements, including group IIB, biennially (organic derivatives of the transition metals are included in other volumes in the Inorganic Chemistry series).

In this volume, chapters devoted to individual metals or related groups of metals review papers published in 1971 and 1972 (for arsenic, antimony and bismuth, early 1971 to March 1973; and for beryllium, magnesium and the alkaline earths, 1969 to 1972). The surveys are inevitably selective, and the judgment of the authors in their choice of significant papers can only be tested by subsequent developments. One omission did seem surprising: although work on various metallocarboranes is reported, there is almost no coverage of carboranes *per se*.

If the unavoidable limitations of this type of survey are accepted, this volume provides a useful key to the original literature, and the price is not excessive. There are, however, some defects, which it is hoped the publishers and editors will remedy in future editions: the apparently random order of the chapters, and the inconsistency in their titles; the brevity of the subject index (and absence of an author index); and the fact that a survey of the literature for 1971–72 has not appeared until August 1975. **B. J. Wakefield**

On-line computing

Laboratory On-Line Computing: An Introduction for Engineers and Physicists. By J. E. Brignell and G. M. Rhodes. Pp. x+297. (International Textbooks: Leighton Buzzard, September 1975.) £8.75.

THIS book sets out to introduce engineers and physicists to on-line computing. The authors wisely confine themselves mostly to generalities but at the same time there is sufficient depth of detail to make this book of real use to persons entering this complex and rapidly developing field.

Two early chapters introduce the reader to the basic elements of both hardware and software, assuming a level of knowledge usually gained in most courses in engineering or physics—a good starting point. But it is in getting to grips with the real problem—hardware-software interaction—that the authors excel, with their ability to delineate and explain the essential features of interfacing. Many complex design features of both hardware and software are

explained with a lucidity which is both refreshing and instructive.

The authors devote a large section of their book to a much neglected subject—the consequences of interfacing the real continuous world to the discretised world of the digital computer. The reader is introduced very gently to theories of sampling and digital filtering.

There are, however, one or two surprising omissions. There is an absence of detailed comment on, for example, either the influence of operating systems on real-time response, or on modern programming techniques which are important for memory-starved minicomputers, such as subroutine re-entrancy. On the hardware side the ubiquitous asynchronous serial line unit is not mentioned.

On the whole, however, this is an excellent book and anyone planning to "go on-line" will certainly find it of great benefit. Research workers and engineers already experienced in on-line computing will find sections which make interesting and valuable reading. **P. F. Kilty**

Polychloroaromatics

Polychloroaromatic Compounds.
Edited by H. Suschitzky. Pp. viii + 543. (Plenum: New York and London, 1975.) \$42.

Most readers will find this book of greatest interest if they read first the last chapter, which is a comprehensive and stimulating survey of polychloroaromatic and -heteroaromatic compounds of industrial importance, by M. B. Green of ICI Mond Division. Insecticides, fungicides, herbicides and others are covered together with certain polymers and dyestuffs. This material gives special point to the earlier chapters.

In the first of these, Ballester and Olivella provide a lengthy account of aromatic and alkaromatic chlorocarbons which is clear, comprehensive and well organised. It contains very interesting material on relevant radicals, reactive and inert, and on carbonium ions and carbanions. It is followed by similar chapters on polychloroheteroaromatic compounds and on polychloroaryl derivatives of metals and metalloids by Iddon and Suschitzky and by Chivers and Wakefield, respectively.

The range of heterocyclic compounds covered is wide, including mainly mono- and bi-cyclic compounds containing one or two heteroatoms (nitrogen, oxygen and sulphur). Similarly, the coverage of metals and metalloids is comprehensive with major emphasis on alkali metals, group II metals, and group IIIA and IVA elements, particularly silicon.

The intrinsic chemical interest attached to all these compounds lies in an attempt to understand their unusual properties in terms of the cumulative electronic effects of the chlorine substituents and corresponding steric effects. Some progress in this direction has been made. The book has a traditional 'preparation and properties' flavour, but is a mine of information, indicative of many problems needing further research.

The book is well conceived and well produced, and each chapter has a full set of references. There is also a satisfactory author and subject index. It is, however, somewhat specialised for most organic chemists. Nevertheless they are all in Professor Suschitzky's debt for making available so conveniently a vast amount of useful and interesting information.

N. B. Chapman

Idealised models

Branching Processes with Biological Applications. (Wiley Series in Probability and Mathematical Statistics.) By P. Jagers. Pp. xiii + 268. (Wiley Interscience: London and New York, October 1975.) £10.50.

ALTHOUGH biological populations often develop in extremely complex patterns, we may imagine idealised situations in which the underlying reproductive process has a simple structure which is free from the usual dependencies of competition, predation and the limitation of natural resources. Such simplified models lend themselves to a mathematical analysis rich in results, and the conclusions drawn from them often give great insight into the behavioural structure of the more complicated processes to which they relate. This mathematical theory has been studied in great generality and the results may be easily interpreted in a practical context.

The basic structure of such idealised models involves the concept of a branching process, in which a population of identical individuals develops in time as the individuals multiply independently of each other and eventually die. As a thorough survey of problems involving multitype branching processes is already in existence (Mode, American Elsevier, New York, 1971), their existence is

mainly avoided in this book.

Within this framework questions may be asked which relate to the probability that a population becomes extinct, to the existence and structure of stable age distributions, to limiting rates of growth, and so on. The general theory surrounding such questions is developed in the first seven chapters, and applications are discussed in the last two. The reader is gently led into the text by way of a historical survey and an analysis of the simplest model available, namely the Galton-Watson process. Generalisations of this special case are considered and the first part of the text concludes with a short chapter on multi-type processes. In a brief interlude the reader is armed with the basic tools of martingales, renewal theory and point processes; and he is then presented with a full treatment of the general theory with all its attendant extensions and ramifications. These complex ideas are then illustrated by reference to several stimulating problems in the fields of demography and cell kinetics.

Dr Jagers writes in an extremely interesting literary style, rare for a mathematician, and his book is a delight to read. A high level of mathematical ability is required for full comprehension of all the theory contained in it, but it is to be hoped that biologists with little mathematical expertise will still find that his conclusions arouse considerable food for thought

E. Renshaw

Proteins

The Proteins. Volume 1. By Hans Neurath, Robert L. Hill and Carol-Leigh Boeder. Third Edition. Pp. xiii + 547. (Academic: New York and London, 1975.) n.p.

THE two previous editions of *The Proteins* have achieved an honoured place in a literature that is fast attaining a complexity matched only by its subject. Is a new edition able to escape the ridicule that in these days such an all-embracing title immediately inspires? Only by the editors quickly informing the reader that generalities and unifying principles will be stressed. Eight volumes are envisaged, of which the first three will deal with general methods, and the remaining five with specific proteins that have unusual structural features or unique biological functions.

This first volume begins well with an admirable account of gel-chromatographic methods of analysis by G. K. Ackers. In view of the extent to which these methods have established themselves in protein chemistry, this seems a proper choice. In the next chapter J. Porath and T. Kristiansen describe the even more significant technique of affinity chromatography, which has achieved a major breakthrough in the last two or three years. The third chapter treats the electrophoresis of proteins in dissociated form in the presence of sodium dodecyl sulphate. A critical account is given by two of the most experienced workers in this field, K. Weber and M. Osborn. In a fourth chapter, K. E. van Holde considers sedimentation analysis. This is a topic where data analysis, and on-line computerisation, is proceeding apace behind the scenes, and we can regard this as a holding exercise for future developments. In chapter five, I. M. Klotz, D. W. Darnall and N. R. Langerman summarise what is known about the quaternary structure of some 300 proteins and describe new methods for chemically cross-linking subunits to detect near neighbours. They also bring the reader into contact with the flourishing study of protein hybrids.

The final chapter on electron microscopy by J. T. Finch provides a very thorough review of the application of image analysis to the determination of the quaternary structure of assemblies of protein molecules.

This is a very good book, written by experts who have had plenty of practice with earlier articles elsewhere.

G. A. Gilbert

Advances in Molten Salt Chemistry, Volume 3. Edited by J. Braunstein, Gleb Mamantov and G. P. Smith. Pp. xi+458. (Plenum: New York and London, 1975.) \$46.20.

"MOLECULAR dynamics calculations of molten ionic salts", by L. V. Woodcock (chapter 1), is a comprehensive review of the methods and application of computer simulation techniques to interpreting the physical chemistry of molten salts. The author concludes that computer simulation offers the only presently viable approach to quantitative interpretation of ionic melt properties. "Gas solubility in molten salts" by P. E. Field reviews the literature up to 1972 and includes results for 14 gases in pure and mixed metal salts of seven anions. "Organic reactions in molten tetrachloroaluminate solvents" by H. L. Jones and R. A. Osteryoung concentrates on condensation-addition reactions, dehydrogenation-addition reactions and rearrangement-isomerisation reactions. The continuing importance of fluorides justifies a major review of "Experimental techniques in molten fluoride chemistry" by C. E. Bamberger. The detailed explanations will be useful to even a beginner in the field.

"The chemistry of thiocyanate melts" by D. H. Kerridge is the first review of the subject, covering thiocyanate melts in most groups of elements. The final chapter (171 pages) by R. E. Thoma describes "Phase diagrams of binary and ternary fluoride systems". This exhaustively catalogues every known phase diagram in which the liquidus is delineated. Hitherto unpublished data from Oak Ridge Laboratory are included, and the publication in English of much Russian literature is invaluable.

This book maintains the high standards of literary and technical excellence which have characterised the series.

H. C. Brookes

Halonium Ions. (Reactive Intermediates in Organic Chemistry.) By George A. Olah. Pp. xiii+190. (Wiley-Interscience: New York and London, November 1975.) \$22.75; £11.40.

THIS new book from Professor Olah is about species of the type RXR^+ , where R and R' may be alkyl or aryl (including cyclic forms), and X a halogen. Aromatic iodonium salts ($\text{ArIAr}^+ \text{X}^-$) were discovered in 1894 and are described in many standard organic texts. Aliphatic halonium ions are more recent and much of the work on them has

emerged from Olah's laboratory in the last decade.

The book describes the preparations, spectroscopy (mainly nuclear magnetic resonance (NMR)) and chemical reactions (alkylation, arylation, nucleophilic attack) of the various types of halonium ion. Stability falls as the groups R change from aromatic to aliphatic and the halogen from iodine to chlorine (fluoronium ions are unknown). The wholly aliphatic RXR^+ ions exist only at low temperatures and there is only NMR evidence (plausible rather than rigorous) for them. Olah is perhaps too certain here; although the balance of probability lies in favour of the halonium ions, more evidence that some of them are not present mainly as carbenium ions is surely needed.

J. Burdon

Books brief

Statistical Prediction Analysis. By J. Aitchison and I. R. Dunsmore. Pp. xi+273. (Cambridge University: Cambridge, London, New York and Melbourne, September 1975.) £8.50.

ONE of the most important problems in applied statistics is that of predicting, on the basis of a number of observations, what a future observation will look like. In its simplest form, $x_1, x_2, \dots, x_n$ are n realisations of a random variable—what can be said about x_{n+1} ? Although study of the problem goes back to Laplace and his law of succession, it does not occupy a prominent position in modern statistical thinking. This is a pity in view of the importance and frequent occurrence of the problem. The present book is a serious and successful attempt to remedy this situation. The introductory chapter contains some excellent examples and later chapters deal with applications to sampling inspection, regulation, optimisation, calibration, diagnosis and treatment allocation. Throughout the book, and particularly in these chapters, the theory is intimately related to practice and non-trivial examples are given in commendable detail. The authors have had to make an agonising decision about the mathematical level of the book. What they have chosen to do is to throw the reader in at the deep end after the gentle introductory chapter. He is sharply immersed in a whole series of distributions, and without some experience of probability distributions he will

certainly be lost. The authors ease his way by full and intelligent tabulation of the distributions and by very clear writing. Within the limits of size they could hardly have done better. The philosophy of the book is largely Bayesian, although there are some deviations in chapters 5 and 6; but I think the reader will be so convinced by the good, sound common sense and practical relevance of the examples that he will scarcely realise that a serious point is at stake. This book is an important contribution to the statistical literature.

D. V. Lindley

Chemistry, Biology, and Clinical Uses of Nucleoside Analogs. (Annals of the New York Academy of Sciences, Volume 255). Edited by A. Bloch. Pp. 610. (New York Academy of Sciences: New York, August 1975.) \$46.00. NUCLEOSIDE analogues have been used with increasing success over the past 20 years in the treatment of cancer and of bacterial viral and parasitic infections. Add to this the clinically useful immunosuppressive properties of some analogues and it is clear that they represent a therapeutically important group of compounds. In addition, some nucleoside analogues have other, perhaps no less important, actions as cardiovascular agents, specific fungicides, radio-protective agents, insect chemosterilants and as regulators of cell function. Some analogues are even growth stimulators (cytokinins), and on a socially responsible note, could, "in the food-short world of tomorrow, conceivably take on great importance". Such is the diversity of the biological properties of the agents described in this volume, that it is now evident that new analogues once isolated or synthesised, should be widely screened, since they may have any one of a number of important properties. Tubercidin (7-deaza-adenine), for instance, was rejected as a clinically useful anti-tubercular or anti-tumour agent because of unacceptable host toxicity. Since no toxicity is encountered on local administration to the skin, however, the agent now has potential value in the treatment of basal cell carcinoma.

This elegantly edited volume is a clear, interesting, and in places fascinating, account of nucleoside analogues, their present day uses and their future. Details are given of new synthetic routes, of the properties of novel analogues and of new ways of using already well-established agents. As the final chapter emphasises, there is still a great deal to be learnt from the study of nucleoside analogues.

T. A. Connors

obituary

Theodosius Grigorievich Dobzhansky, world renowned population geneticist and evolutionist, died on December 18, 1975 in Davis, California. Correspondence concerning journal articles received by his colleagues suggest that he was working until the moment of death.

Dobzhansky was born on January 25, 1900 at Nemirov, near Kiev, in the USSR, where he studied biology at the University. After graduating, he became a Lecturer in Genetics at the University of Leningrad. In 1927 he received a Rockefeller Foundation fellowship to study with T. H. Morgan at Columbia University and at the California Institute of Technology, when Morgan moved there. Dobzhansky joined the faculty of the Institute after the expiry of his fellowship. He remained there as Professor of Genetics until he returned to Columbia University in 1940. In 1962 Dobzhansky joined the research staff of the Rockefeller Institute (later Rockefeller University) in New York City. On his retirement in 1971, he moved to the University of California at Davis, where he worked until his death.

Early in his career, Dobzhansky ('Dodik' or, as his American friends came to call him, 'Doby') led an expedition into central Asia studying the genetics of domestic animals. He developed a lifelong passion for horse-back riding during that trip. Subsequently, nearly every site for field studies was selected to include accessibility to a riding stable; a ride of several hours on a 'splendid animal' was an essential feature of his daily routine.

Until joining Morgan's group, Dobzhansky spent some ten years studying

the taxonomy, geographic variation, and polymorphism of coccinellid (lady-bird) beetles. These studies, influenced by his former professor, S. S. Chetverikov, laid the foundation upon which he built his later theories of the origin of geographic races.

After joining Morgan's group, Dobzhansky started his work on *Drosophila*. He commenced a number of studies that would now be regarded as 'classical' genetics: in respect to the question of the pleiotropic (multiple) action of genes, he showed that a number of common mutant alleles in *D. melanogaster* had consistent effects on internal organs as well. His work on the location of gene loci through the use of chromosomal translocations led to the first cytological maps of gene loci. He pursued the genetics of sex-determination and intersexuality in *Drosophila*, and made valuable contributions to the then-baffling phenomenon of 'position effect'.

Dobzhansky's work on the population and evolutionary genetics of *D. pseudo-obscura* began in the early 1930s with studies on the hybrid sterility of 'inter-racial' crosses. After discovering a wealth of chromosomal inversions in these flies, he recognised that the order in which these inversions occurred could be deduced from present day gene arrangements. A phylogeny of chromosomal rearrangements spanning three species was eventually constructed.

The studies on the genetics of natural populations of *D. pseudo-obscura* and other *Drosophila* species were reported in a series of 43 papers, the last of which appears in the March 1976 issue of *Genetics*. These studies also formed

the basis of Dobzhansky's classic text, '*Genetics and the Origin of Species*' (retitled '*Genetics of the Evolutionary Process*' in its 1970 edition). He constantly related knowledge gained firsthand from his beloved flies and beetles to appropriate biological aspects of human beings; out of such efforts came '*Mankind Evolving*', a text that is often favourably compared with his earlier classic.

Dobzhansky will influence the course of population genetics for years to come. In addition to the 34 or so doctoral students whom he supervised, his laboratory was host to a phalanx of postdoctoral fellows from all parts of the world. At last count, Dobzhansky had been the recipient of 21 honorary degrees.

In concluding this review of Dobzhansky's many accomplishments, it is necessary to mention the vigour with which he opposed those whom he perceived as charlatans. For many years, he attacked Lysenko and Lysenkoism as that man and his followers sought to destroy both genetics and the geneticists within Stalin's USSR. Similarly, he criticised outspokenly the pseudo-science of those in the United States and elsewhere who preached the superiority of one racial group over another. And he had no patience with those who, on religious grounds, oppose evolution and the teaching of evolution in the public schools. Weakened by age and terminal disease, Dobzhansky could nevertheless muster the strength needed to thunder at a carping anti-evolutionist, "SIR! I do not question your honesty! I question your INTELLIGENCE!"

Lee Ehrman
Bruce Wallace

Professor Sir Alexander Haddow died on January 21, three days after his 69th birthday. Honours, high academic distinction and recognition of his work came to him from many institutions and countries, and these and the list of his numerous offices can be found in reference books. This is a more personal tribute and an account of his 40 years devoted to the Research Institute of the Royal Cancer Hospital (Free), known, after moving to new premises, as the Chester Beatty Research Institute.

In 1935 he arrived there from Edinburgh to join Kennaway, and in 1946 succeeded Kennaway as Director. The

run-down inevitably associated with the war was reversed and he initiated a ten-year period of growth and expansion. He insisted that cancer research must not be regarded in any narrow light but must be pursued as with basic science, to which it, in turn, had to contribute. He thought himself "an anti-planner and an anti-organisation man" and he would have been appalled to hear himself described as a good administrator, yet he was just that. He provided an organisation which facilitated research, yet one that was quite free from bureaucratic complexity and he saw to it that no-one was diverted from what he described as 'their proper

labour' by administrative tasks.

To achieve this took much time and effort but yet it did not stop Alex Haddow from pursuing his own research work, and from guiding the work of a large team. His knowledge of all aspects of cancer research was impressive and he took immense pains to help and advise. He never forced his own views, yet a large part of the work of the Institute, past and present, can be traced both to his guidance, enthusiasm and, not infrequently, to his own research discoveries. His attitude to research was that it was an intensely individual pursuit and that workers must be left to develop their own

promise: the discipline was personal and that imposed by the subject. He impressed on all that while work had to be carefully planned, it must not be rigid to exclude or overlook the unanticipated chance or the unintended discovery which he considered to be one of the principal objectives of cancer research.

He gave up the directorship in 1969 because of his failing eyesight and retired as Professor of Experimental Pathology three years later. He did not allow this affliction to stop his work and he was active until a few weeks before his death.

His own research work, which covered a vast spectrum from the first properly conducted clinical trial of a cancer chemotherapeutic agent to studies of drug action at the molecular level, was dominated by the concept that there is a link between impairment of growth and the eventual induction of cancer. Early in his career he found that chemicals which induce cancer are almost invariably toxic to dividing cells, both normal and malignant. The full implications of this

observation for the mechanisms of carcinogenesis have yet to be revealed, but for the development of the modern era of cancer chemotherapy this finding was crucial. The study of the mode of action of carcinogens and of anti-cancer agents was seen by him to be closely associated, and led to the discovery within the Institute of some of the most valuable and widely used cancer chemotherapeutic agents.

While Haddow foresaw long before most the clinical possibilities of cancer chemotherapy, he appreciated the limitations of the available compounds and insisted that research efforts must be concentrated on their mode of action in the hope of achieving greater specificity. His belief that, in spite of the great diversity of carcinogens, there must be an underlying unity of mechanism and that a truly comprehensive synthesis is possible has been fully validated by recent findings that carcinogens of the widest heterogeneity induce, by various routes, very similar biochemical lesions.

His personality was complex but the key to it was his intense concern for

people and causes. His idealism extended into many fields and he found the time to give active support to bodies involved with the prevention of nuclear war and the misuse of science in all its aspects, such as Pugwash. He wrote cogently on all these topics and to foster them accepted a seat on the Press Council and the advisory council of the B.B.C. He also contributed greatly to the international organisation of cancer research, both through the International Union, in which he served as President, and by personal contact.

His concern for people determined the atmosphere of the Institute. He was tolerant, generous, had an intense interest in the well-being of all who worked for him and with him and went to endless trouble to help with professional and personal problems. Haddow's immense mental and physical energy and his versatility derived from his conviction that problems intellectual, medical or social were capable of being solved and he had no sympathy for those who skirted their direct confrontation.

Peter Alexander

announcements

International meetings

March 31–April 2. **The magnetosphere/Particle physics**, Sheffield (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1 8QX, UK).

April 2. **Chemically Active Species in Biology**, The London School of Hygiene and Tropical Medicine (Assistant Secretary, The Society of Chemical Industry, 14 Belgrave Square, London SW1X 8PS).

April 8–9. **Stress in Animals on Experiment**, a Symposium organised by LASA and BLAVA, Guildford (Dr P. Eaton, Charing Cross Hospital Medical School, Fulham Palace Road, London W6 9HH).

June 7–9. **Chlorophyll-Proteins Reaction Centres and Photosynthetic Membranes**, Brookhaven (Deadline for Contributions, April 16) (Dr John M. Olson, Biology Department, Brookhaven National Laboratory, Upton, New York 11973).

June 23–25. **First International Symposium on HLA and Disease**, Paris (Deadline for Abstracts, April 28 to Professor J. Dausset, Centre Hayem, Hôpital St-Louis, 75475 Paris) (Congrès-Services, 1 rue Jules Lefebvre, 75009 Paris, France).

July 19–22. **Fifth International Symposium on Medicinal Chemistry**, Paris (Congress secretariate: 49 rue St-

Person to Person

Some members of the Department of Mathematics at Edinburgh University, believe that there is scope for improved co-operation between mathematicians and R and D workers in industry, and a group has been formed to identify and work on industrial problems. They wish to hear of suitable problems and to meet representatives of the firms concerned to discuss possible co-operation.

Any problem submitted would be discussed at first by the group as a whole to decide whether the requisite skills are available. The problem would then become the main concern of one or more members, with the active support of the rest of the group for discussions and workshops.

Enquiries to The Director, Centre for Industrial Consultancy and Liaison, University of Edinburgh, 14 George Square, Edinburgh EH8 9JZ, UK.

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

Andre-des-Arts, 75006 Paris).

October 27. **Ultrasonic Spectroscopy**, London (Papers: Professor A. F. Brown, The City University, St John's Street, London EC1V 4PB; Information: The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

Reports and publications

Other countries

Unesco. Intergovernmental Oceanographic Commission. Technical Series No. 11: Bruun Memorial Lectures. (Presented at the Eighth Session of the IOC Assembly, Unesco, Paris 5–17 November 1975.) Pp. 63. (Paris: Unesco, 1975.) [812]

The Coconut Industry Board, Jamaica. 14th Report of the Research Department, July 1973/June 1974. Pp. 64. (Kingston, Jamaica: Coconut Industry Board, PO Box 204, 1975.) J\$3; £1.50; US\$3. [912]

World Health Organization: Regional Office for Europe, Copenhagen. Report of the Regional Director, July 1974 to June 1975. Pp. 105. (Copenhagen: WHO, Regional Office for Europe, 1975.) [912]

CERN—European Organisation for Nuclear Research. CERN 75–15: Terminals for the Interactive Input and Editing of Bibliographic Records on the PDP-11 Computer in the CERN Library. By A. Van Praag. Pp. 60. CERN 75–16: Effects of Nuclear Radiation on the Optical Properties of Cerium-Doped Glass. By B. McGrath, H. Schonbacher and M. Van de Voorde. Pp. 7. CERN 75–17: Calculation and Optimization of Study Fields of Septum Dipole Magnets. By A. J. T. Holmes. Pp. 19. (Geneva: CERN, 1975.) [1012]

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Chemical Physics, 1974/1975. Pp. 78. Division of Chemical Physics—Research and Facilities. Pp. 90. (Clayton, Victoria, Australia: Division of Chemical Physics, CSIRO, 1975.) [1112]

Australia: CSIRO. Bulletin No. 288: Energetics of Agriculture and Food Production. By Roger M. Gifford and R. J. Millington. Pp. 29. (Melbourne: CSIRO, 1975.) [1112]

Australia: CSIRO. Division of Applied Geomechanics—Research Report 1975. Pp. 90. (Mount Waverley, Victoria: Division of Applied Mechanics, CSIRO, 1975.) [1112]